COMPOSITIONS AND METHODS FOR TREATING BACTERIAL INFECTIONS WITH PROTEIN-DALBAVANCIN COMPLEXES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application Serial Nos. 60/427,654, filed November 18, 2002, 60/485,694, filed July 8, 2003, 60/495,048, filed August 13, 2003, and 60/496,483, filed August 19, 2003, the disclosures of all of which are incorporated herein by reference in their entireties.

BACKGROUND OF THE INVENTION

Field of the Invention

[0002] This invention is directed to complexes of dalbavancin with endogenous proteins. These complexes can be formed *in vivo*, *in vitro*,or *ex vivo* and provide for an antimicrobial effect in a treated patient.

State of the Art

[0003] According to the U.S. Center for Disease Control and Prevention, nosocomial bloodstream infections are a leading cause of death in the United States. Approximately five percent of the seven million central venous catheters (CVCs) inserted annually in the United States are associated with at least one episode of bloodstream infection (approximately 350,000 a year). Catheter-related bloodstream infections occur when bacteria enter the bloodstream through an intravenous catheter and can be life threatening.

[0004] Skin and soft tissue infections (SSTIs) are a common medical condition and often the consequence of trauma or surgical procedures. *Staphylococcus aureus* and *Streptococcus pyogenes* are the pathogens most frequently isolated from patients with deep tissue infections, although any pathogenic organism, including those found on healthy skin, may cause infection. Many SSTIs are mild to moderate in severity, permitting successful treatment with oral antimicrobial agents and local cleansing. In contrast, more severe or complicated infections, which frequently occur in patients with underlying risk factors (*e.g.*, vascular compromise, diabetes) and/or infections caused by difficult-to-treat or multiply-resistant bacteria, may require potent intravenous antimicrobial therapy and aggressive surgical debridement.

[0005] Staphylococci are a clinical and therapeutic problem and have been increasingly associated with nosocomial infections since the early 1960s. The coagulase-positive species methicillin-resistant *Staphylococcus aureus* (MRSA) has long been problematic in both community-acquired and nosocomial infections, and several coagulase-negative staphylococci have been recognized as opportunistic human pathogens, especially in the treatment of critically ill patients in intensive care units. Another major cause for clinical concern is the increasing isolation of penicillin-resistant *Streptococcus pneumoniae* strains in many parts of the world.

[0006] The glycopeptide antibiotics vancomycin and teicoplanin have been used against serious nosocomial infections caused by multi-drug-resistant Gram-positive pathogens, particularly MRSA, coagulase-negative staphylococci (CoNS), and enterococci. Vancomycin and teicoplanin are used for infections caused by MRSA, and until recently, all isolates were uniformly susceptible. However, the isolation of *Staphylococcus aureus* strains with intermediate susceptibility or resistance to teicoplanin as well as vancomycin has now been reported with increasing frequency. A number of vancomycin-resistant strains, classified "VanA," "VanB," or "VanC," based on the mechanism of resistance, have been reported. Thus, alternative treatment options are needed.

[0007] Teicoplanin is at least as active as vancomycin against most Gram-positive bacteria and appears to cause fewer adverse events. Both forms of treatment require at least once daily dosing to effect complete recovery. Currently, the therapeutic options for severe infections caused by some of these pathogens is quite limited. The emerging resistance of Gram-positive pathogens to vancomycin makes the availability of new antibiotics with potential for increased effectiveness highly desirable.

[0008] In addition, less frequent dosing regimens than currently-available therapies would be desirable to enhance patient comfort, especially for parenteral, *e.g.,* intravenous or intramuscular, antibiotic administration. Hospital stays are sometimes necessitated by the need for multi-daily antibiotic administration by parenteral means, and less frequent dosing would be advantageous to permit such treatment to be done on an outpatient basis.

[0009] Although less frequent dosing is a desirable feature of an antibiotic administration regimen, the "pharmaceutical window," *i.e.*, the toxicity profile, of the administered antibiotic must be sufficiently acceptable to permit a large single dose to be administered without jeopardizing treatment by causing severe adverse reactions in the treated patient. Further, even when an antibiotic exhibits a suitable pharmaceutical window, less frequent dosing is possible only if the

antibiotic exhibits a suitable serum half-life to maintain therapeutic effectiveness over the dosing interval desired. The serum half-life of an antibiotic dictates both the longevity of a drug *in vivo* and the length of time after administration when the serum level will reach a minimum trough level which is still bactericidally effective. The serum trough level over time after administration of a first dose of antibiotic dictates when a further dose must be administered to retain a minimum bactericidal level of the antibiotic *in vivo*.

[0010] In view of the above, an antibiotic possessing activity against one or more antibiotic resistant bacterial strains, particularly MRSA, which could be administered at a dosing interval of once every 5-7 days or longer, would be of commercial value and would satisfy a long-felt need in the art.

SUMMARY OF THE INVENTION

[0011] This invention is directed to the discovery that upon administration *in vivo*, dalbavancin forms a complex with endogenous proteins, *e.g.*, human serum albumin (HSA). Studies indicate that there are two separate binding sites on HSA and that the formed complex can be either in a 1:1 ratio of dalbavancin to HSA or a 2:1 ratio. The particular complex formed will, of course, be dependent upon the amount of dalbavancin administered, the rate of administration, and the total amount of endogenous protein available for complexation.

[0012] This dalbavancin-protein complex formed *in vivo* retains dalbavancin antibacterial properties and exhibits a long serum half-life. Contrarily, many other antibiotic-protein complexes either lose potency upon complex formation and/or the complex is readily cleared from the body thereby limiting the duration of antibacterial activity.

[0013] Still further, the formation of the complex permits systemic tissue distribution of the dalbavancin in a treated patient which is essential to the successful treatment of skin and soft tissue infections.

[0014] Without being limited to any theory, it is believed that, *in vivo*, this complex either exhibits antibacterial properties *per se* or acts as a drug depot wherein the low binding affinity of dalbavancin to the endogenous protein permits sustained release of free dalbavancin over a period of time. In either case, a prolonged antibacterial effect is achieved.

[0015] Accordingly, in one of its composition aspects, this invention is directed to an endogenous protein-dalbavancin complex comprising both a 1:1 complex of protein to dalbavancin and a 1:2 complex of protein to dalbavancin.

[0016] The dalbavancin component may contain one or more of the A_o, A_1, B_o, B_1, and MAG dalbavancin components. Preferably, the dalbavancin component in the complex comprises at least about 80% $B_{o;}$ more preferably, from at least about 80% to about 98% of the B_o component.

[0017] In one embodiment, the complex is formed *in vivo* by intravenous administration of a dalbavancin composition to the patient. In a preferred embodiment, the ratio of 1:1 and 1:2 complexes of protein to dalbavancin

[0018] is controlled by the amount and rate of intravenous administration of the dalbavancin composition to the patient. Preferably, the administration is conducted under conditions such that the initial plasma concentration of dalbavancin is at least 200 mg/L and at least about 90% of the complex formed has a 1:1 ratio of dalbavancin to protein.

[0019] Accordingly, in another of its composition aspects, this invention is directed to a dalbavancin-endogenous protein complex formed *in vivo* by intravenous administration of a dalbavancin composition to a mammalian patient under conditions wherein the initial plasma concentration of dalbavancin is at least 200 mg/L and further wherein that least about 90% of the complex formed has a ratio of dalbavancin to protein of 1:1.

[0020] Alternatively, the dalbavancin-endogenous protein complex can be formed *in vitro* or *ex vivo* by admixing a solution of dalbavancin with protein under conditions wherein the complex is formed. When so formed, this invention further provides for pharmaceutical compositions comprising a pharmaceutically acceptable carrier and an antibacterial amount of the complex. Such compositions may be sterile and/ore lyophilized and/or in pharmaceutically acceptable form for administration to an individual, e.g., in a pharmaceutically acceptable aqueous formulation. In embodiments, the individual is a mammal, e.g., a human.

[0021] In some embodiments, the invention provides pharmaceutical compositions that include a pharmaceutically acceptable carrier, a protein-dalbavancin complex of the invention, and a non-dalbavancin antibiotic or mixture of non-dalbavancin antibiotics. The non-dalbavancin antibiotic or mixture of antibiotics may include at least one antibiotic that is effective against a Gram negative bacterium.

[0022] In a preferred embodiment, a stabilizer is employed in the dalbavancin composition used to form the complex described herein. The stabilizer is employed to inhibit degradation of one or more dalbavancin components during storage whether as a lyophilized or aqueous formulation. Over time, degradation can result in the undesirable formation of less active/inactive components in the dalbavancin composition or in the formation of components which potentially could cause adverse side effects *in vivo*. Particularly preferred stabilizers are non-ionic components such as a sugar or sugar alcohol, *e.g.*, a mono-, di-, or polysaccharide, or derivative thereof, such as, for example, mannitol, lactose, sucrose, sorbitol, glycerol, cellulose, trehalose, or maltose, or mixtures thereof.

[0023] In some embodiments, protein-dalbavancin complexes provided by the invention retain at least about 10% of the antibacterial of free dalbavancin. In some embodiments, protein-dalbavancin complexes provided by the invention permit systemic distribution of dalbavancin in an individual when present in the individual.

[0024] In one of its method aspects, this invention is directed to a method for treating a bacterial infection in an individual in need thereof, said method comprising administering a therapeutically effective dose of dalbavancin to an individual suffering from a bacterial infection under conditions such that a protein-dalbavancin complex forms.

[0025] In a particularly preferred embodiment, there is provide a method for treating a bacterial infection in an individual in need thereof. This method comprises administering a sufficient dose of dalbavancin to the individual to provide an initial plasma concentration of dalbavancin of at least about 200 mg/L, wherein dalbavancin forms a protein-dalbavancin complex with an endogenous protein, and further wherein that least about 90% of the complex formed has a ratio of dalbavancin to protein of 1:1.

[0026] In another method aspect, this invention is directed to a method for treating a bacterial infection in an individual in need thereof, comprising administering a therapeutically effective dose of a protein-dalbavancin complex.

[0027] In some embodiments of therapeutic method aspects of the invention, the therapeutically effective dose contains an amount of dalbavancin or protein-dalbavancin complex sufficient to provide a therapeutically effective serum level in said individual for at least 5 days. In some embodiments, a first and second therapeutically effective dose of dalbavancin or protein-dalbavancin complex are administered, where the amount of the second dose is about half or less

than the amount of the first dose. In some embodiments, the dose of dalbavancin is about 500 mg to about 1000 mg. In some embodiments, the bacterial infection comprises a Gram-positive bacterium, e.g. a penicillin-resistant or multi-drug-resistant bacterium. In some embodiments, the bacterial infection comprises a skin and soft tissue infection (SSTI); in some of these embodiments, the SSTI comprises *Staphylococcus aureus,* in other embodiments the SSTI comprises *Streptococcus pyogenes*. In some therapeutic method embodiments, the bacterial infection is reduced; in some embodiments it is eliminated. In some therapeutic method embodiments, the individual is a mammal, *e.g.*, a human.

[0028] In a further method aspect, this invention is directed to a method for preventing onset of a bacterial infection in an individual, comprising administering dalbavancin in a prophylactically effective dose under conditions such that a protein-dalbavancin complex forms.

[0029] In a yet further method aspect, this invention is directed to a method for preventing onset of a bacterial infection in an individual, comprising administering a protein-dalbavancin complex in a prophylactically effective dose.

[0030] In prevention methods of the invention, the administration of dalbavancin or protein-dalbavancin complex occurs prior to, during, or subsequent to a medical procedure; e.g., surgery, or insertion of an intravenous catheter. In some embodiments, the administration of dalbavancin or protein-dalbavancin complex occurs prior to, during, or subsequent to a stay in the hospital.

[0031] In some embodiments of preventive method aspects of the invention, the prophylactically effective dose contains an amount of dalbavancin or protein-dalbavancin complex sufficient to provide a prophylactically effective serum level in said individual for at least 5 days. In some embodiments, a first and second prophylactically effective dose of dalbavancin or protein-dalbavancin complex are administered, where the amount of the second dose is about half or less than the amount of the first dose. In some embodiments, dalbavancin or protein-dalbavancin complex is administered as a single dose that comprises about 250 mg to about 1000 mg of dalbavancin.

[0032] In all method aspects, administration may be parenteral. *e.g.*, intravenous, e.g., controlled intravenous administration. In some embodiments, controlled intravenous administration occurs over at least about thirty minutes. In some embodiments, the methods further comprise administering a pharmaceutically acceptable carrier. In some embodiments, the methods further comprise administering a non-dalbavancin antibiotic or mixture of non-dalbavancin antibiotics. In

some of these embodiments, at least one of the non-dalbavancin antibiotics is effective against a Gram negative bacterium to the individual.

[0033] In another aspect, the invention provides kits containing dalbavancin or a protein-dalbavancin complex, and instructions for use, where the use may be in methods of treatment or methods of prevention of a bacterial infection.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] **Figure 1** depicts dalbavancin plasma concentration versus time following a single 1000 mg intravenous infusion of dalbavancin.

[0035] **Figure 2** depicts isothermal titration calorimetry data for dalbavancin binding to human serum albumin (top) and a graphical representation of the data fitted to a curve determined from a 2:1 binding model of dalbavancin:protein (bottom).

[0036] **Figure 3** depicts an electrospray ionization mass spectrum of dalbavancin.

[0037] **Figure 4** is a graph of dalbavancin concentration vs. population ratio of dalbavancin multimer to monomer and depicts an increase in population ratio of dalbavancin multimer to monomer with increasing dalbavancin concentration.

[0038] **Figure 5** is a graph of pH vs. population ratio of dalbavancin multimer to monomer and depicts an increase in population ratio of dalbavancin multimer to monomer with increasing pH.

[0039] **Figure 6** depicts an electrospray ionization mass spectrum of dalbavancin in an ammonium formate 5mM pH 5 solution.

[0040] **Figure 7** depicts an electrospray ionization mass spectrum of dalbavancin in an ammonium formate 50 mM pH 5 solution.

[0041] **Figure 8** depicts an electrospray ionization mass spectrum of dalbavancin in an ammonium formate 100 mM pH 5 solution.

[0042] **Figure 9** depicts an electrospray ionization mass spectrum of teicoplanin (50 μg/mL) in water.

[0043] **Figure 10** depicts an electrospray ionization mass spectrum of teicoplanin (100 μg/mL) in water.

[0044] **Figure 11** depicts the effect of HSA on the apparent dissociation constant for dalbavancin/tri-peptide binding at 26°C (pH 7.4).

[0045] **Figure 12** depicts a comparison of isothermal calorimetry (ITC) data for binding of tri-peptide to vancomycin and dalbavancin under identical conditions, using the same tri-peptide solution.

[0046] **Figure 13 (A and B)** depicts the possible interaction of dalbavancin monomers and multimers (including dimers) with tri-peptide ligand and HSA. Figure 13(A) depicts dalbavancin in monomer-dimer equilibrium in solution, binding as monomer to two separate sites on HSA; Figure 13(B) depicts ligand binding to dalbavancin dimer in solution and more weakly to dalbavancin monomers attached to HSA.

DETAILED DESCRIPTION OF THE INVENTION

[0047] The present invention provides protein-dalbavancin complexes, pharmaceutical compositions of protein-dalbavancin complexes, and improved methods of treatment of antibiotic-resistant bacterial infections. In particular, this invention provides for dalbavancin compositions having activity against one or more antibiotic resistant strains of bacteria and particularly MRSA, which compositions can be administered on a dosing regimen of once every 5-7 days or longer.

[0048] Dalbavancin, which is also referred to in the scientific literature as BI 397 or VER001, is a semi-synthetic glycopeptide mixture, the properties of which have been reported in U.S. Pat. Nos. 5,606,036, 5,750,509, 5,843,679, and 5,935,238.

[0049] As used herein, the term "dalbavancin" refers to compositions comprising one or more, preferably two or more, closely related homologs, termed "A_0," "A_1," "B_0," "B_1," "C_0," and "C_1," as described below, or monomers, multimers (*i.e.*, dimer or higher order multimer), tautomers, esters, solvates, or pharmaceutically acceptable salts thereof. As used herein, "dimer" or "multimer" refers to either a homodimer or homomultimer, *i.e.*, a dimer or multimer composed of monomers of the same dalbavancin homolog, or a heterodimer or heteromultimer, *i.e.*, a dimer or multimer composed of monomers of at least two different dalbavancin homologs. Dalbavancin often includes "MAG," a non-homolog variant described below. Individually, dalbavancin homologs and MAG are sometimes referred to herein as "dalbavancin components."

[0050] Dalbavancin is prepared by chemical modification of the natural glycopeptide complex A-40,926 as described in Malabarba and Donadio (1999) *Drugs of the Future* 24(8):839-846. The

predominant component of dalbavancin is Factor B_0, which accounts for >75% of the whole complex.

[0051] The amount of each of the components present in a dalbavancin composition is dictated by a variety of factors, including, for example, the fermentation conditions employed in the preparation of the natural glycopeptide complex A-40926, which is the precursor to dalbavancin (see, *e.g.*, U.S. Pat. No. 5,843,679), the conditions employed to recover A-40926 from the fermentation broth, the chemical reactions employed to selectively esterify the carboxyl group of the sugar moiety of A-40926, the conditions employed to amidate the peptidyl carboxyl group, the conditions employed to saponify the ester of the carboxyl group of the N-acylaminoglucuronic acid function, the conditions employed to recover dalbavancin from the synthetic mixture, and the like.

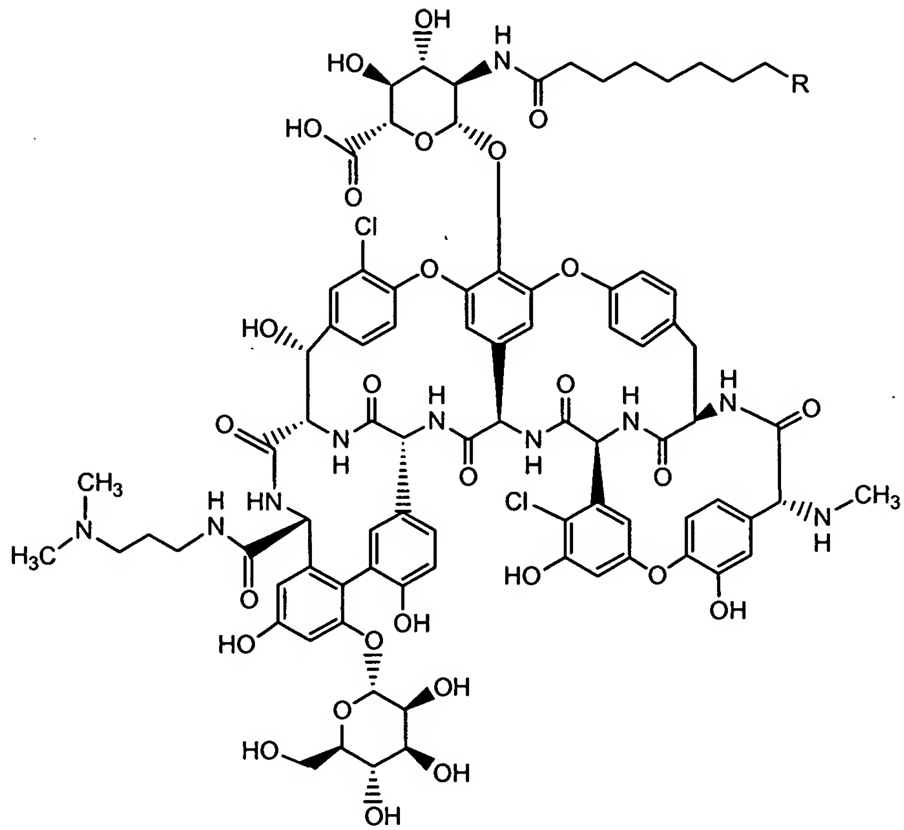
[0052] In preferred embodiments, dalbavancin compositions comprise at least about 80 to about 98% by weight of the B_0 component. In particularly preferred embodiments, dalbavancin comprises the following amounts of B_0:

Table 1. Preferred Amounts of B_0 Component in Dalbavancin Composition

Preferred[1]	More Preferred[1]	Even More Preferred[1]
80-98	80-97	80-96
81-98	81-97	81-96
82-98	82-97	82-96
83-98	83-97	83-96
84-98	84-97	84-96
85-98	85-97	85-96
86-98	86-97	86-96
87-98	87-97	87-96
88-98	88-97	88-96
89-98	89-97	89-96
90-98	90-97	90-96

1 each range represents the mole % of B_o relative to the total dalbavancin_components present in the dalbavancin composition including MAG

[0053] The chemical structure of several of the dalbavancin components is depicted in Formula I below:



Dalbavancin Component	R	Molecular Weight
A_o	$-CH(CH_3)_2$	1802.7
A_1	$-CH_2CH_2CH_3$	1802.7
B_o	$-CH_2CH(CH_3)_2$	1816.7
B_1	$-CH_2CH_2CH_2CH_3$	1816.7
C_0	$-CH_2CH_2CH(CH_3)_2$	1830.7
C_1	$-CH_2CH_2CH_2CH_2CH_3$	1830.7

[0054] All of the above dalbavancin components are bactericidally active against a number of Gram-positive bacteria. However, one non-homologous dalbavancin component, termed "MAG," which lacks an acylglucoronamine moiety present in other components, is less bactericidally effective, both *in vivo* and *in vitro*, than other dalbavancin components. MAG is thought to be a decomposition product of one or more of the other dalbavancin components. Accordingly, in a

preferred embodiment, the amount of MAG in dalbavancin is less than about 4, 3.5, 3, 2.5, 2, 1.5, 1, or 0.5 mole percent of all dalbavancin components present, including MAG.

[0055] Dalbavancin is thought to inhibit the biosynthesis of the bacterial cell wall by binding to D-alanyl-D-alanine-terminating precursors of peptidoglycans. Dimeric or higher order multimers of dalbavancin may possess further antibacterial properties by interaction of the lipophilic side chains with the cytoplasmic membrane of bacteria. See, for example, Malabarba and Ciabatti, et al. (2001) *Current Medicinal Chemistry* 8:1759-1773. A further elaboration on dalbavancin multimers may be found in U.S. Serial No. 10/______, entitled "DALBAVANCIN COMPOSITIONS FOR TREATMENT OF BACTERIAL INFECTIONS," filed concurrently herewith as Attorney Docket No. 34231-20052.00, the disclosure of which is hereby incorporated by reference in its entirety.

[0056] *In vitro*, nonclinical, and clinical data indicate dalbavancin to be of benefit for the treatment of serious Gram-positive infections caused by MRSA and CoNS, and all streptococcal and non-VanA enterococcal species, including VanB and VanC phenotypes poorly susceptible or resistant to vancomycin.

[0057] Dalbavancin is more active *in vitro* against staphylococci (including some teicoplanin-resistant strains) than teicoplanin and vancomycin. Dalbavancin has better activity against streptococci, including penicillin-resistant strains, than teicoplanin or vancomycin. Dalbavancin is active *in vitro* and *in vivo* against a number of Gram-positive bacteria, including most drug resistant strains.

[0058] Dalbavancin is typically administered to an individual as a dalbavancin composition. As used herein, the term "dalbavancin composition" or "dalbavancin formulation" refers to a composition, typically a pharmaceutical composition comprising dalbavancin, as defined above, and one or more other non-dalbavancin components such as, for example, a pharmaceutically acceptable carrier, a stabilizer, a buffers, or other similar components.

[0059] As shown in Example 1, dalbavancin is effective at dose intervals of one week. Thus, an advantage of dalbavancin versus other treatment options is the ability to administer this antibiotic on a once-weekly basis, thereby maximizing patient compliance and potentially minimizing the need for or decreasing the length of a hospital stay for parenteral antibiotic administration. Less frequent dosing often permits treatment on an out-patient basis, thus decreasing treatment costs. As further shown in Example 1, a second dose of dalbavancin approximately one week after

administration of the first dosage, where the second dose is approximately one-half the first dose, unexpectedly provides significant improvement in the efficacy of treatment.

[0060] Surprisingly, dalbavancin has been found to form complexes with proteins, *e.g.*, endogenous proteins, that provide a substantial retention of dalbavancin antibacterial activity and that allow systemic tissue distribution of dalbavancin in a mammal to unexpectedly high levels.

Protein-dalbavancin complex

[0061] The invention provides protein-dalbavancin complexes that retain physiological antibacterial activity, *e.g.*, the ability to reduce or eliminate a bacterial infection. The protein-dalbavancin complexes of the invention further permit systemic tissue distribution of dalbavancin. As used herein, "protein-dalbavancin complex" refers to a protein molecule bound to at least one dalbavancin molecule. Some embodiments of the invention refer to a plurality of such complexes. The binding may be non-covalent or covalent. However, without being limited to any theory, it is believed that the complex constitutes non-covalent binding of one or more molecules of a dalbavancin component to the protein and, as such, the binding may be ionic and/or hydrophobic in nature.

[0062] The complex may be formed *in vivo, in vitro,* or *ex vivo*. Often the complex comprises dalbavancin monomer; often, the complex includes a dalbavancin multimer, *e.g.*, a dalbavancin dimer. The ratio of protein molecule to dalbavancin molecule is generally 1:1 (one dalbavancin molecule per protein molecule) or 0.5:1 (two dalbavancin molecules per protein molecule), although lower ratios are encompassed by the invention. When the ratio of protein molecule to dalbavancin molecule is 0.5:1, the two dalbavancin molecules may bind to the protein as two monomers or as a single dimer. However, again without being limited to any theory, it is believed that the 0.5:1 complex constitutes two molecules of a dalbavancin component in monomeric form bound to different sites on the protein.

[0063] The dalbavancin component(s) of the complex may be any of the components of dalbavancin; if more than one dalbavancin component is bound to the protein, each component may be the same or different from the other component(s). Some embodiments provide a plurality of protein-dalbavancin complexes, where the B_o component of dalbavancin accounts for at least 90% of the dalbavancin molecules (components) in the complexes.

[0064] Some embodiments of the invention provide a plurality of protein-dalbavancin complexes that contain at least about 80, 85, 90, 95, or 98 % B_o, relative to the total dalbavancin in the complexes, either in an individual in which complexes form *in vivo*, or in a composition prepared *ex vivo* or *in vitro*. In one embodiment, the protein-dalbavancin complexes contain at least 90% of the B_o homolog. Preferably, the amount of MAG in the total complexes, relative to overall dalbavancin content, is less than about 4, 3.5, 3, 2.5, 2, 1.5, 1, or 0.5% MAG. MAG does not bind substantially to some proteins useful in the protein-dalbavancin complexes of the invention; *e.g.*, isothermal calorimetry data indicate that MAG does not bind substantially to human serum albumin. Without being limited to theory, this is consistent with a mechanism of binding of dalbavancin to HSA primarily involves the fatty acid side chain group. Percentages in all cases are mole percent.

[0065] The dalbavancin of the protein-dalbavancin complexes of the invention may comprise a pharmaceutically acceptable, non-toxic salt of dalbavancin. Examples of suitable salts of dalbavancin include salts formed by standard reaction with both organic and inorganic acids such as, for example, hydrochloric, hydrobromic, sulfuric, phosphoric, acetic, trifluoroacetic, trichloroacetic, succinic, citric, ascorbic, lactic, maleic, glutamic, camphoric, glutaric, glycolic, phthalic, tartaric, lauric, stearic, salicylic, methanesulfonic, benzenesulfonic, sorbic, picric, benzoic, cinnamic, and the like acids. Representative examples of bases that can form salts with dalbavancin include alkali metal or alkaline earth metal hydroxides such as sodium, potassium, calcium, magnesium, and barium hydroxide, ammonia and aliphatic, alicyclic, or aromatic organic amines such as methylamine, dimethylamine, diethylamine, ethanolamine, and picoline. (See, for example, U.S. Pat. No. 5,606,036 which is incorporated herein by reference in its entirety.) Dalbavancin is often provided for formation of a protein-dalbancin complex as a hydrochloride salt, which is freely soluble in water.

[0066] The "protein" of the protein-dalbavancin complex may be an endogenous protein. As used herein, "endogenous protein" refers to a protein (*i.e.*, a type of protein, for example, serum albumin) produced by an individual to whom dalbavancin is administered, or a modified form thereof. Modified forms of endogenous proteins include synthetically or recombinantly produced variants. Modified forms of endogenous proteins may include functionalities with improved binding to dalbavancin, improved stability *in vivo* and/or *in vitro*, and/or enhanced antibacterial activity when complexed with dalbavancin. In some embodiments, the endogenous protein is an albumin, such as, for example, human serum albumin. For *in vitro* complex formation, an

"endogenous protein" may be extracted from an individual to whom dalbavancin is to be administered, extracted from another individual, or may be produced recombinantly and/or via chemical synthesis. Thus, "endogenous protein," as used herein, refers to a protein type, such as human serum albumin, that is endogenous to the individual to whom dalbavancin is to be administered, whether the source of the protein is, *e.g.*, the individual, another individual, or a recombinant source. Recombinant human serum albumin suitable for pharmaceutical administration is described in, *e.g.*, U.S. Pat. Nos. 5,962,649; 5,986,062; and 6,617,133 which patents are incorporated herein by reference in their entirety. Typically, an "endogenous protein" does not produce an immune response in an individual sufficient to significantly reduce antibacterial effectiveness of dalbavancin.

[0067] The protein-dalbavancin complexes of the invention may contain any ratio of protein molecule and dalbavancin molecules. In some complexes, the ratio of protein molecules to dalbavancin molecules is 1:1. In some complexes, the ratio of protein molecule to dalbavancin molecules is about 0.5:1, *i.e.*, two dalbavancin molecules per protein molecule. In the latter complexes, the dalbavancin molecules may bind to the protein as a dimer, or as two monomers. In embodiments of the invention in which the protein-dalbavancin complex forms *in vivo*, complexes with a ratio of protein to dalbavancin of 1:1 comprise about 90%, 95%, or 99%, of the total protein-dalbavancin complexes in an individual. In embodiments of the invention in which the protein-dalbavancin complex is formed *ex vivo* or *in vitro*, and where the ratio of protein to dalbavancin is subject to manipulation, complexes with a ratio of protein to dalbavancin of 1:1 comprise about 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99% of the total protein-dalbavancin complexes. In all cases, the remainder of the complexes contain a ratio of protein molecule to dalbavancin of less than 1:1, *e.g.* 0.5:1 (two dalbavancin molecules per protein molecule).

[0068] The protein:dalbavancin ratio may be measured by means known in the art. To determine the presence and/or amount of protein-dalbavancin complex *in vitro* or *in vivo*, an exemplary method is to find the difference between total dalbavancin and free dalabavancin. Total dalbavancin may be measured by means described herein. Free dalbavancin may be determined by, *e.g.*, microdialysis or ultracentrifugation. Alternatively, electrophoresis, with, *e.g.*, labeled dalbavancin, may be used to directly measure protein-dalbavancin complexes.

[0069] A protein-dalbavancin complex may be formed *in vivo*, by administration of dalbavancin to an individual, or *ex vivo* or *in vitro*, by contacting dalbavancin and a protein, *e.g.*, an endogenous

protein, under suitable conditions. The complex may be formed *ex vivo* or *in vitro* prior to administration or may be formed *in vivo* upon administration of dalbavancin. In some methods in which the complex is formed *in vivo*, the complex is formed upon controlled administration of dalbavancin. In some embodiments, the complex is formed upon intravenous administration of dalbavancin over a time period of at least about 30 minutes duration.

[0070] Protein-dalbavancin complexes are formed *in vivo* administering a suitable dose of dalbavancin to an individual, *e.g.* in a human, about 200 mg, 500 mg, 1000 mg, or more than about 1000 mg of dalbavancin, at a controlled rate, *e.g.*, by controlled intravenous infusion, over a period of about 10, 20, 30, or more than about 30 minutes. In one embodiment of the invention, protein-dalbavancin complexes, in which at least about 90%, or at least about 95%, of the complexes have a ratio of protein molecules to dalbavancin molecules of 1:1, are formed *in vivo* by controlled intravenous infusion to a human of a dose of dalbavancin of about 1000 mg over a time period of at least about 30 minutes, so that a serum concentration of dalbavancin (*i.e.*, free dalbavancin and dalbavancin in a complex) of at least about 200 mg/L is reached.

[0071] A protein-dalbavancin complex may also be formed *in vitro*, and either stored in solution, often as a sterile pharmaceutical composition, or lyophilized under conditions in which stability of the complex is retained. If desired, a solution or lyophilized composition including a protein-dalbavancin complex may be sterilized, for example, by e-beam or gamma sterilization methods, or by sterile filtration. Heat sterilization may be used provided there is no denaturation of the protein and/or loss of antibacterial properties.

[0072] *In vitro* formation may be achieved by contacting the dalbavancin and protein, *e.g.*, an endogenous protein, under suitable conditions so that complexes with the desired ratio of dalbavancin components, as well as the desired ratio of protein molecules to dalbavancin, are formed. The ratio of individual components of dalbavancin may be modulated by adjusting the ratios of individual components in the dalbavancin used to form the complexes. In embodiments of the invention, dalbavancin that comprises at least about 90%, 95%, or 98% B_o component, is used in the preparation of the protein-dalbavancin complexes. In some embodiments, dalbavancin that comprises between about 80% and about 98% Bo is used in the preparation of the protein-dalbavancin complexes. The ratio of protein molecules to dalbavancin molecules in the protein-dalbavancin complexes may be modulated by adjusting the molar ratio of protein and dalbavancin in the mixture in which complexes are formed. In general, a ratio of protein (*e.g.*, HSA) to

dalbavancin of 1:1 or more results in protein-dalbavancin complexes in which the percentage of complexes where the ratio of protein molecule to dalbavancin is 1:1 is at least 90%, and generally at least 95%. The protein used in *in vitro* formation may be any protein that does not produce an immunological response in the individual to whom it will be delivered sufficient to interfere with the antibacterial effect of dalbavancin. Generally, the protein may be any endogenous serum protein, *e.g.*, α-1 glycoprotein or serum albumin (*e.g.*, human serum albumin). In some embodiments, the protein is an endogenous protein and is human serum albumin, and in some of these embodiments the human serum albumin is recombinant human serum albumin, produced by means known in the art. See, *e.g.*, U.S. Pat. Nos. 5,962,649; 5,986,062; and 6,617,133 which patents are incorporated herein by reference in their entirety.

[0073] For *ex vivo* formation, blood from an individual is removed, and endogenous protein or a mixture of endogenous proteins is obtained from the blood by means known in the art and contacted with dalbavancin under conditions where protein-dalbavancin complexes of the desired mixture of dalbavancin components and ratio of dalbavancin to protein is formed. Conditions for such formation are as described for *in vitro* formation.

[0074] It is contemplated that a protein-dalbavancin complex typically will retain-physiological antibacterial activity for at least 5 days, often at least one week, sometimes at least 15 days, *in vitro*. In methods for treating an individual with a bacterial infection, a plasma level of dalbavancin in a protein-dalbavancin complex is often maintained at a therapeutically effective level of at least about 4, 5, 10, 20, or 30 mg dalbavancin per liter for at least about 5 days.

[0075] A striking feature of the protein-dalbavancin complexes of the invention is their retention of the antibacterial effect of dalbavancin. Dalbavancin in serum may be more than 95% protein-bound, but its *in vivo* activity greatly exceeds what would be expected for a compound with a free fraction of less than 5%. In embodiments of the invention, the protein-dalbavancin complex retains at least about 5%, 10%, 15%, 20%, 30%, 40%, 50%, or more than 50% of the antibacterial activity of free dalbavancin at the same concentration, *in vitro* or *in vivo*. Another striking feature of the protein-dalbavancin complexes of the invention is that, despite the high percentage of dalbavancin that can exist as a complex with protein, the drug is widely distributed, *i.e.*, its volume of distribution *in vivo* approximates extracellular water volume.

Methods of use

[0076] Methods are provided for administration of dalbavancin to an individual in need of treatment for a bacterial infection under conditions where a protein-dalbavancin complex forms. Methods are also provided for administration of a protein-dalbavancin complex to an individual in need of treatment for a bacterial infection. Treatment can include prophylaxis, therapy, or cure. Methods include administration of one or more unit doses of dalbavancin in a therapeutically or prophylactically effective amount. Methods also include administration of non-dalbavancin antibiotics in addition to dalbavancin or protein-dalbavancin complex.

[0077] As used herein, "therapeutically effective amount" refers to the amount of protein-dalbavancin complex that will render a desired therapeutic outcome (*e.g.*, reduction or elimination of a bacterial infection). A therapeutically effective amount may be formed by administration of one or more doses of dalbavancin, or may be administered as one or more doses of protein-dalbavancin complex. A "prophylactically effective amount" refers to an amount of protein-dalbavancin complex sufficient to prevent or reduce severity of a future bacterial infection when formed in or administered to an individual who is susceptible to and/or who may contract a bacterial infection by virtue of a medical procedure or stay in the hospital, or exposure to an individual with a bacterial infection. Dalbavancin or protein-dalbavancin complex is generally administered in a pharmaceutically acceptable carrier. Dalbavancin is often provided as a hydrochloride salt, which is freely soluble in water.

[0078] As used herein, "individual" refers to a vertebrate, typically a mammal, often a human.

[0079] Typically, dalbavancin or protein-dalbavancin complex is administered as a "unit dose" which includes an amount of dalbavancin sufficient to provide a therapeutically or prophylactically effective plasma level of dalbavancin for several days, often at least about 2 days, 5 days, one week, or 10 days, when administered to an individual. The novel dosage regimen available for dalbavancin and protein-dalbavancin complexes results in improved efficacy because blood levels of dalbavancin are maintained above minimum bactericidal levels for the entire treatment protocol.

[0080] All homologs of dalbavancin described above exhibit a prolonged half-life in plasma, often 9 days or more, although MAG is thought to have a shorter half-life than other homologs. The long half-life permits longer intervals between dosages than vancomycin or teicoplanin. As described in Example 1, weekly dosing of dalbavancin is effective for control of bacterial infections, in contrast to the twice daily dosing schedule which is often used for vancomycin or the

once daily schedule generally used for teicoplanin. Less frequent dosing of dalbavancin offers significant treatment advantages over vancomycin and teicoplanin, particularly with regard to improved convenience and patient compliance with the treatment regimen. Surprisingly high doses (*i.e.*, resulting in surprising high and long-lasting serum levels) can be administered, and with less frequency than other available treatment options. The novel dosage regimen available for dalbavancin results in improved efficacy because at concentrations required to effect less frequent dosing, dalbavancin exhibits minimal adverse effects *in vivo*, evidencing a large pharmaceutical window, and further because blood levels of dalbavancin are maintained above minimum bactericidal levels for the entire treatment protocol, evidencing a prolonged serum half-life for dalbavancin. The combination of the large pharmaceutical window coupled with prolonged serum half-life permits less frequent dosing of dalbavancin.

[0081] In addition, dalbavancin is preferably formulated with a stabilizer which inhibits degradation of one or more of the components of dalbavancin. In one preferred embodiment, dalbavancin is formulated with a 1:2 weight ratio of mannitol:dalbavancin. In another preferred embodiment, dalbavancin is formulated with a 1:1:4 weight ratio of mannitol:lactose:dalbavancin.

[0082] In some embodiments, dalbavancin is administered under conditions such that a dalbavancin-protein complex forms, and at a dosage that results in therapeutically effective (*e.g.*, bactericidal) plasma levels of the drug for several days, often at least about 5 to about 10 days, often at least about one week. In some embodiments, protein-dalbavancin complex is administered at a dosage that results in therapeutically effective (*e.g.*, bactericidal) plasma levels of the drug for several days, often at least about 5 to about 10 days, often at least about one week. In either embodiment, generally, dalbavancin is maintained in plasma at or above the minimum bactericidal concentration of about 4 mg/l for at least 5 days. Often, dalbavancin is maintained at a plasma level of at least about 5 mg/l, often at least about 10 mg/l, often at least about 20 mg/l, often at least about 30 mg/l, often at least about 40 mg/l, for at least 5 days, often at least about one week or longer. Plasma levels of dalbavancin may be measured by methods that are well known in the art, such as liquid chromatography, mass spectrometry, or microbiological bioassay. An example of a method for quantitating dalbavancin in plasma is provided in Example 5. Upper limits for dalbavancin plasma concentration levels are generally dictated by dosages which inhibit unacceptable adverse effects in the patient population treated.

[0083] In some embodiments, dalbavancin may be administered, under conditions where protein-dalbavancin complexes form, in a single dose or in multiple doses. In some embodiments, dalbavancin is administered, under conditions where protein-dalbavancin complexes form, weekly for two or more weeks. In one embodiment, dalbavancin is administered, under conditions where protein-dalbavancin complexes form, in at least two doses, often in two doses about 5 to about 10 days apart, more often once a week for two weeks. Dalbavancin also may be administered, under conditions where protein-dalbavancin complexes form, in multiple doses two or more days or at least one week apart or in one or more biweekly doses. In some embodiments, dalbavancin is administered, under conditions where protein-dalbavancin complexes form, weekly, followed by biweekly, or monthly administration. In some embodiments, dalbavancin is administered, under conditions where protein-dalbavancin complexes form, at weekly intervals for 2, 3, 4, 5, 6, or more weeks.

[0084] In some embodiments, protein-dalbavancin complex may be administered in a single dose or in multiple doses. In some embodiments, protein-dalbavancin complex is administered weekly for two or more weeks. In one embodiment, protein-dalbavancin complex is administered in at least two doses, often in two doses about 5 to about 10 days apart, more often once a week for two weeks. Protein-dalbavancin complex also may be administered in multiple doses two or more days or at least one week apart or in one or more biweekly doses. In some embodiments, protein-dalbavancin complex is administered weekly, followed by biweekly, or monthly administration. In some embodiments, protein-dalbavancin complex is administered at weekly intervals for 2, 3, 4, 5, 6, or more weeks.

[0085] In embodiments where dalbavancin is administered under conditions where protein-dalbavancin complexes form, or where protein-dalbavancin complex is administered, most advantageously daily dosing is not required because higher, less frequent doses are used. Single or multiple doses may range in dalbavancin content, for example, from about 0.1 to about 5 grams. A single dose with a dalbavancin content of about 0.1 to about 4 grams, *e.g.*, about 3 grams, may be administered for various infection treatments. Where multiple doses are administered, for example, weekly, each dose may range in dalbavancin content, for example, from about 0.25 to about 5.0 grams. As used herein, "dalbavancin content" of a dose refers to the amount of dalbavancin present whether administered as free dalbavancin or as part of a protein-dalbavancin complex, or as a mixture of the two.

[0086] For embodiments in which a single dose is administered to treat an infection, the dalbavancin content of the dose may be, for example, about 0.1 to about 5 grams, or about 0.5 to about 4 grams, or about 1 to about 3.5 grams, or about 2 to about 3 grams *e.g.*, about 3 grams. In some embodiments, a single dose containing about 1, 1.5, 2, 2.5, or 3 grams of dalbavancin is administered for treatment of a bacterial infection. For embodiments in which a single dose of dalbavancin or protein-dalbavancin complex is administered for prophylaxis, the dalbavancin content of the dose may be, for example, about 0.1 to about 3 grams, or about 0.1 to about 1 gram, *e.g.*, about 0.5 or about 0.25 gram.

[0087] In dosing schemes that include multiple dosages, the individual dosages may be the same or different. Each dose may be given in the form of free dalbavancin (under conditions where a protein-dalbavancin complex forms), or in the form of protein-dalbavancin complex, or a mixture of the two. In some embodiments, a first, higher dose is administered, that is, for example, about 1.5 to 3 times higher in dalbavancin content, than one or more subsequent doses. For example, the first dose may contain about 0.5 grams to about 5 grams and the second dose about 0.25 grams to about 2.5 grams, the first dose may contain about 0.8 to about 2 g and the second dose about 0.4 to about 1 gram, or the first dose may contain about 0.4 to about 3 g and the second dose about 0.2 to 1.5 g.

[0088] In some embodiments, at least two dosages are administered wherein the first dosage includes about twice as much dalbavancin as subsequent dosages. In one embodiment, a first dosage contains about 1 gram of dalbavancin and a subsequent dosage contains about 0.5 gram. In another embodiment, a first dosage contains about 0.5 gram of dalbavancin and a subsequent dosage contains about 0.25 gram.

[0089] In some embodiments, dalbavancin or protein-dalbavancin complex is administered in two doses of equal or different amount two or more days or at least about one week apart. Often, two doses containing about 0.2 to about 1.5 grams of dalbavancin are administered about 5 to about 10 days apart, more often about 1 week apart. In one embodiment, a first dosage containing about 1 gram of dalbavancin and a second dosage containing about 0.5 gram of dalbavancin are administered about 1 week apart.

[0090] In a multiple dosing regimen, the time between doses may range, for example, from about 5 to about 10 days, often about one week. Dose frequency may be, for example, two weekly doses, or multiple weekly doses. The dosing interval, or time between doses, can be, for example,

any of about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more days. The number of doses given, can be, for example, one, two, three, four, five, six or more doses, each dose after the initial dose being given after the selected dosage interval.

[0091] In a multiple dosing scheme, often the "trough level," or the level of dalbavancin in plasma after a first dose of dalbavancin or protein-dalbavancin complex and just prior to administration of a second dose, is at least about 4 mg/l. Preferably, the trough level at the end of a dosing interval such as about one week is at least about 10 mg/l, more preferably at least about 20 mg/l, more preferably at least about 30 mg/l, and even more preferably at least about 40 mg/l.

[0092] Dalbavancin or protein-dalbavancin complex can be administered parenterally, *e.g.*, intramuscularly (i.m.), intravenously (i.v.), subcutaneously (s.c.), intraperitoneally (i.p.), or intrathecally (i.t.). The dosing schedule and actual dosage administered may vary depending on such factors as the nature and severity of the infection, the age, weight, and general health of the patient and the tolerance of a particular patient to dalbavancin or protein-dalbavancin complex, but will be ascertainable to health professionals. In one embodiment, an intravenous dose containing one gram of dalbavancin is followed by an intravenous dose containing 0.5 gram dalbavancin one week later.

[0093] Administration and delivery of dalbavancin or protein-dalbavancin complex to the patient, *e.g.*, intravenously, can be done at a controlled rate, so that the concentration in the blood does not increase too quickly or cause precipitation to occur. The infusion duration can be, for example, about 1 minute to about 2 hours. For example, an infusion duration of about 30 minutes may be used where the dose contains about 0.5 to about 1 gram dalbavancin. When the dalbavancin is given in the form of protein-dalbavancin complex, the infusion duration for a dose containing about 0.5 to about 1 gram of dalbavancin may be shorter, *e.g.*, about 1, 2, 5, 10, 15, 20, or 25 minutes. Intravenous administration of dalbavancin or protein-dalbavancin complex under controlled rate conditions can generate concentrations of dalbavancin in the body that are in great excess of what can be achieved at physiological pH *in vitro*.

[0094] The content of dalbavancin administered may be any of the dosages disclosed herein. The dalbavancin dose is generally chosen such that the drug will remain at a therapeutically or prophylactically effective (*i.e.*, bactericidal) plasma level for an extended period of time, often at least 5 days, more often about one week or longer. Administration of a dose of dalbavancin or protein-dalbavancin complex which produces and maintains bactericidal concentrations for at least

about one week (or about 5 to about 10 days) is preferred. A bactericidal concentration is defined as the concentration of dalbavancin required to kill at least 99% of the bacteria present at the initiation of an *in vitro* experiment over a 24 hour period. A minimum bactericidal concentration of dalbavancin in plasma is typically about 4 mg/l.

[0095] Examples of indications that can be treated include both complicated and uncomplicated skin and soft tissue infections (SSTI), blood stream infections (BSI), catheter-related blood stream infections (CRBSI), osteomyelitis, prosthetic joint infections, surgical prophylaxis, endocarditis, hospital or community acquired pneumonia, pneumococcal pneumonia, empiric treatment of febrile neutropenia, joint space infections, and device infections (*e.g.*, pace makers and internal cardiac defibrillators). Gram-positive or antibiotic-resistant bacterial infections may be treated, such as a *Staphylococcus, Streptococcus, Neisseria*, or *Clostridium* genus infection, in particular *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus hemolyticus, Streptococcus pyogenes, Groups A and C Streptococcus, Neisseria gonorrhoeae,* or *Clostridium difficile.*

[0096] The invention provides methods for treatment of skin and soft tissue infections (SSTIs). Patients who may benefit from this treatment may have either deep or superficial infections. SSTI may involve deeper soft tissue and/or require significant surgical intervention, such as for example a major abscess, infected ulcer, major burn, or deep and extensive cellulitis. Infected surgical wounds may also be treated.

[0097] The clinical presentation of skin and skin structure infection may vary from mild folliculitis to severe necrotizing fasciitis. The mode of acquisition may also vary with community-acquired skin and skin structure infections, which are often preceded by injuries resulting from occupational exposure or recreational activities, and are usually associated with a greater diversity of pathogens. Hospital-acquired skin and skin structure infections are generally associated with surgical procedures, the development of pressure sores, and catheterization. Post-surgical infections are the third most frequent nosocomial infection and account for 17% of all nosocomial infections reported to the National Nosocomial Infection Surveillance System (NNIS). The most frequent source of infection is the patient's endogenous flora. *Staphylococcus aureus*, coagulase-negative staphylococci, and *Enterococcus spp.* are the pathogens most frequently isolated from SSTIs.

[0098] Symptoms of SSTI infections may include erythema, tenderness or pain, heat or localized warmth, drainage or discharge, swelling or induration, redness, or fluctuance. Patients that may benefit from treatment with the methods of the invention include those with deep or

complicated infections or infections that require surgical intervention, or patients with underlying diabetes mellitus or peripheral vascular disease. These infections are often caused by Gram-positive bacteria such as *Staphylococcus* or *Streptococcus* species, such as *Staphylococcus aureus* or *Streptococcus pyogenes*. Methods for treatment of a skin or soft tissue bacterial infection include administering a therapeutically effective amount of dalbavancin to an individual in need of treatment, in a form (*i.e.*, dalbavancin or protein-dalbavancin complex), amount, and according to a dosing regime as discussed above. In some embodiments, dalbavancin or protein-dalbavancin complex is administered intravenously in two doses, often about 5 to about 10 days apart, more often about 1 week apart. In some embodiments, the first dosage contains at least twice as much dalbavancin as the second dosage. In one embodiment, the first dosage contains about 1000 mg of dalbavancin and the second dosage contains about 500 mg of dalbavancin.

[0099] The invention also provides methods for prophylactic prevention of the onset of a bacterial infection, for example an infection caused by *Staphylococcus aureus*, or by a *Neisseria* or *Clostridium* genus bacterium. In a prophylactic method of the invention, a prophylactically effective amount of dalbavancin or protein-dalbavancin complex is administered to an individual who may be susceptible to contracting a bacterial infection, for example through a medical procedure. Often, dalbavancin or protein-dalbavancin complex is administered in an amount sufficient to provide a prophylactically effective plasma level of dalbavancin for at least about 3 days, at least about 5 days, or at least about one week or longer. Dalbavancin or protein-dalbavancin complex may be administered, for example, parenterally, *e.g.*, via intramuscular (i.m), intravenous (i.v.), intraperitoneal (i.p.), subcutaneous (s.c.), or intrathecal (i.t.) injection, prior or subsequent to surgery as a preventative step against infection. Dalbavancin or protein-dalbavancin complex may be administered immediately prior or subsequently to, 1 or more days or about one week prior or subsequently to, or during an invasive medical procedure such as surgery or a stay in a medical care facility such as a hospital to prevent infection. A prophylactic method may be used in any situation in which it is possible or likely that an individual may contract a bacterial infection, including situations in which an individual has been exposed to or is likely to be exposed to a bacterially infected individual. For prophylactic methods, dalbavancin or protein-dalbavancin complex may be administered as either a single dose or as two or more doses of equal or different amount that are administered several days to about one week apart. In one embodiment,

dalbavancin or protein-dalbavancin complex may be administered prior to or simultaneously with insertion of an intravenous catheter in order to prevent a bloodstream related infection.

[0100] For prophylactic methods, dalbavancin or protein-dalbavancin complex may be administered in a single dose or in multiple doses, according to any of the dosing schemes described above. Often, dalbavancin or protein-dalbavancin complex is administered as a single dose containing about 0.1 to about 3 grams of dalbavancin, or about 0.1 to about 1 gram, *e.g.*, about 0.25 gram or about 0.5 gram. In one embodiment, a single dose containing about 0.25 gram is administered intravenously over a time frame of about 2 minutes to about 1 hour, *e.g.*, about 30 minutes. In another embodiment, dalbavancin or protein-dalbavancin complex is administered intravenously simultaneously with administration of another pharmaceutical (*e.g.*, antibiotic) treatment.

[0101] In any of the therapeutic or prophylactic methods described above, dalbavancin or protein-dalbavancin complex may be administered either simultaneously or sequentially with at least one other antibiotic. In some embodiments, at least one other antibiotic that is bactericidally effective against one or more Gram-negative bacterial species and/or a Gram-positive bacterial strain against which dalbavancin is not effective is administered in addition to dalbavancin or protein-dalbavancin complex. In some embodiments, dalbavancin or protein-dalbavancin complex and at least one antibiotic that is bactericidally effective against at least one Gram-negative bacterial species is administered as a mixture.

Pharmaceutical compositions

[0102] The invention provides pharmaceutical compositions formulated for administration of dalbavancin or protein-dalbavancin complex according to the methods described above. Pharmaceutical compositions of the invention may be in the form of a unit dose of dalbavancin or protein-dalbavancin complex that contains an amount of dalbavancin sufficient to provide a therapeutically or prophylactically effective plasma level of dalbavancin for several days, often at least about 3 days, at least about 5 days, or at least about one week or longer when the composition is administered to an individual, and a pharmaceutically acceptable carrier. Generally, a therapeutically or prophylactically effective plasma level of dalbavancin is at least about 4 mg per

liter of plasma. Plasma levels of dalbavancin may be measured by well known methods in the art, such as those described above.

[0103] Dalbavancin or the dalbavancin component of a protein-dalbavancin complex may optionally be in a pharmaceutically acceptable form for administration to an individual, optionally as a pharmaceutically acceptable, non-toxic salt.

[0104] Examples of suitable salts of dalbavancin include salts formed by standard reaction with both organic and inorganic acids such as, for example, hydrochloric, hydrobromic, sulfuric, phosphoric, acetic, trifluoroacetic, trichloroacetic, succinic, citric, ascorbic, lactic, maleic, glutamic, camphoric, glutaric, glycolic, phthalic, tartaric, lauric, stearic, salicylic, methanesulfonic, benzenesulfonic, sorbic, picric, benzoic, cinnamic, and the like acids. Representative examples of bases that can form salts with dalbavancin include alkali metal or alkaline earth metal hydroxides such as sodium, potassium, calcium, magnesium, and barium hydroxide, ammonia and aliphatic, alicyclic, or aromatic organic amines such as methylamine, dimethylamine, diethylamine, ethanolamine, and picoline. (See, for example, U.S. Pat. No. 5,606,036.)

[0105] In some embodiments, a pharmaceutically acceptable aqueous formulation of dalbavancin or protein-dalbavancin complex is provided that is suitable for parenteral administration, such as, for example, intravenous injection. For preparing such an aqueous formulation, methods well known in the art may be used, and any pharmaceutically acceptable carriers, diluents, excipients, or other additives normally used in the art may be used.

[0106] A pharmaceutical composition for parenteral administration includes dalbavancin or protein-dalbavancin complex and a physiologically acceptable diluent such as deionized water, physiological saline, 5% dextrose, water miscible solvent (*e.g.*, ethyl alcohol, polyethylene glycol, propylene glycol, etc.), non-aqueous vehicle (*e.g.*, oil such as corn oil, cottonseed oil, peanut oil, and sesame oil), or other commonly used diluent. The formulation may additionally include a solubilizing agent such as polyethylene glycol, polypropylene glycol, or other known solubilizing agent, buffers for stabilizing the solution (*e.g.*, citrates, acetates, and phosphates) and/or antioxidants (*e.g.*, ascorbic acid or sodium bisulfite). (See, for example, U.S. Patent No. 6,143,739.) Other suitable pharmaceutical carriers and their formulations are described in "Remington's Pharmaceutical Sciences" by E. W. Martin. As is known in the art, pharmaceutical preparations of the invention may also be prepared to contain acceptable levels of particulates (e.g.,

particle-free) and to be non-pyrogenic. (e.g., meeting the requirements of an injectable in the U.S. Pharmacopeia).

[0107] In one embodiment, a pharmaceutical composition is provided by dissolving a dried (e.g., lyophilized) dose of dalbavancin, often containing a stabilizer or mixture of stabilizers, in an amount of water and preferably deionized water in a volume sufficient for solubilization. Typically, the amount of water sufficient for solubilization is approximately 10 mL and the resulting pH of the dalbavancin solution is above 3.0, and about 3.5 to 4.5. Diluting this solution by adding it to a second amount of an aqueous diluent, often containing 5% dextrose, such as an amount of contained in a drip bag for intravenous administration, raises the pH of the dalbavacin solution to about 5 to 5.5. In another embodiment, the pH of the dalbavancin solution in a drip bag is about 4.5. The second amount of aqueous solution may be deionized or sterile, or both deionized and sterile.

[0108] Pharmaceutical compositions for parenteral administration may be made up in sterile vials containing one or more unit doses of dalbavancin or protein-dalbavancin complex in a therapeutically or prophylactically effective amount as described above, optionally including an excipient, under conditions in which bactericidal effectiveness of dalbavancin is retained. The composition may be in the form of a dry (e.g., lyophilized) powder. Prior to use, a physiologically acceptable diluent may be added and the solution withdrawn via syringe for administration to a patient. A pharmaceutical formulation as described above may be sterilized by any acceptable means including, for example, by e-beam or gamma sterilization methods, or by sterile filtration.

[0109] A typical formulation for parenteral administration may contain dalbavancin, alone or as part of a protein-dalbavancin complex, at a concentration such as about 0.1 to about 100 mg, about 0.5 to about 50 mg, about 1 to about 10 mg, or about 2 to about 4 mg of dalbavancin per ml of final preparation.

[0110] In some embodiments, a pharmaceutical composition in accordance with the invention includes a mixture of dalbavancin or protein-dalbavancin complex and one or more additional antibiotics. Preferably, at least one non-dalbavancin antibiotic in the mixture is bactericidally effective against one or more species of Gram-negative bacteria and/or one or more Gram-positive bacterial strains against which dalbavancin is not effective. The mixture may also include a pharmaceutically acceptable carrier as described above.

[0111] In some embodiments, pharmaceutical compositions of the invention include one or more stabilizing substances which inhibit degradation of one or more of the components of

dalbavancin to less active or inactive materials. As used herein, "stabilizing substance" or "stabilizer" refers to a substance that stabilizes the level of one or more of the constituent components of dalbavancin, for example, B_0, in the composition. A "stabilizing effective amount" refers to an amount of a stabilizer sufficient to enhance long-term stability of one or more components of a dalbavancin composition. In some embodiments, a stabilizing effective amount may be provided by a mixture of two or more stabilizing substances, each of which alone is not present in an amount sufficient to provide a stabilizing effect.

[0112] Examples of stabilizers include, for example, nonionic substances such as sugars, *e.g.*, mono-, di-, or polysaccharides, or derivatives thereof, sugar alcohols, or polyols. Such stabilizing substances include, for example, mannitol, lactose, sucrose, sorbitol, glycerol, cellulose, trehalose, maltose, raffinose, or mixtures thereof.

[0113] In one embodiment, the pharmaceutical composition includes a weight ratio of 1:2 mannitol:dalbavancin. In another embodiment, the pharmaceutical composition includes a weight ratio of 1:1:4 mannitol:lactose:dalbavancin. Often, the pH of a pharmaceutical composition of the invention is, for example, about 3 to about 5, for example about 3.5 or about 4.5.

[0114] In some embodiments, one or more procedures may be employed to reduce formation of MAG. For example, freeze drying of dalbavancin in the presence of a stabilizing substance, such as mannitol, may be employed to reduce the amount of MAG formed.

[0115] Storage of dalbavancin compositions is often at lower than ambient temperature, such as at about 5° C, to enhance stability.

Kits

[0116] The invention also provides kits for use in methods of treatment or prophylaxis of bacterial infections. The kits include a pharmaceutical composition of the invention, for example including at least one unit dose of dalbavancin or protein-dalbavancin complex, and instructions providing information to a health care provider regarding usage for treating or preventing a bacterial infection. Instructions may be provided in printed form or in the form of an electronic medium such as a floppy disc, CD, or DVD, or in the form of a website address where such instructions may be obtained. Instructions may include information on administration conditions for dalbavancin that lead to the formation of a protein-dalbavancin complex. Often, a unit dose of dalbavancin or

protein-dalbavancin complex includes a dosage such that when administered to an individual, a therapeutically or prophylactically effective plasma level of dalbavancin is maintained in the individual for at least 5 days. In some embodiments, a kit includes two unit dosages to be administered at least 5 days apart, often about one week apart, often including a first dosage of dalbavancin or protein-dalbavancin complex that is about 1.5 to about 3 times higher than the second dosage. Dalbavancin or protein-dalbavancin complex is often included as a sterile aqueous pharmaceutical composition or lyophilized composition.

[0117] Kits of the invention may include, in addition to dalbavancin or protein-dalbavancin complex, a non-dalbavancin antibiotic or mixture of non-dalbavancin antibiotics, for use with dalbavancin or protein-dalbavancin complex as described in the methods above.

[0118] Instructions in the kits of the invention may include information on preparation and administration of the kit contents (*e.g.*, dalbavancin or protein-dalbavancin complex, and optionally non-dalbavancin antibiotics), possible adverse reactions, recommended dosages, dosing schedules, and the like. In some embodiments, instructions may describe, for example, administration of dalbavancin under conditions so that a protein-dalbavancin complex forms, in order to treat or to prevent a bacterial infection. In some embodiments, instructions may describe, for example, administration of protein-dalbavancin complex in order to treat or prevent a bacterial infection. In some embodiments, instructions may describe, for example, administration of dalbavancin under conditions sush that a protein-dalbavancin complex forms, and simultaneous or sequential administration of non-dalbavancin antibiotics, in order to treat or to prevent a bacterial infection. In some embodiments, instructions may describe, for example, administration of protein-dalbavancin complex, and simultaneous or sequential administration of non-dalbavancin antibiotics, in order to treat or to prevent a bacterial infection.

[0119] Suitable packaging is provided. As used herein, "packaging" refers to a solid matrix or material customarily used in a system and capable of holding within fixed limits a dalbavancin or protein-dalbavancin complex composition suitable for administration to an individual. Such materials include glass and plastic (*e.g.*, polyethylene, polypropylene, and polycarbonate) bottles, vials, paper, plastic, and plastic-foil laminated envelopes and the like. If e-beam sterilization techniques are employed, the packaging should have sufficiently low density to permit sterilization of the contents.

[0120] Kits may also optionally include equipment for administration of dalbavancin or protein-dalbavancin complex, such as, for example, syringes or equipment for intravenous administration, and/or a sterile buffered solution for preparing a lyophilized composition for administration.

[0121] In the examples below, the following abbreviations have the following meanings. If an abbreviation is not defined, it has its generally accepted meaning.

AcOH	=	acetic acid
AcONa	=	sodium acetate
ALT	=	amino alanine transferase
AST	=	aspartate amino transferase
aq.	=	aqueous
BV	=	bed volume
CV	=	coefficient of variation
d	=	diameter
D	=	dalton
DCC	=	dicyclohexylcarbodiammide
DMEPA	=	3-(dimethylamino)-propylamine
DMSO	=	dimethyl sulfonamide
eq	=	equivalents
EU	=	endotoxin units
g	=	gram
GC	=	gas chromatography
HCl	=	hydrochloric acid
H_2O	=	water
HOBT	=	1-hydroxybenzothiazole hydrate
HPLC	=	high performance liquid chromatography
H_2SO_4	=	sulfuric acid
IPA	=	isopropylamine
IU	=	international unit
KF	=	potassium fluoride
Kg	=	kilogram
L	=	liter
LSC	=	liquid scintillation counting
LDH	=	lactate dehydrogenase
m^3	=	cubic meter
MeOH	=	methanol
mg	=	milligram
mL	=	milliliter
mol	=	molar
MW	=	molecular weight
N	=	normal
NaOH	=	sodium hydroxide
NMP	=	N-methyl-2-pyrrolidone
QTD	=	qualitative tissue distribution

Rt	=	retention time
SD	=	standard deviation
TEA	=	triethylamine

[0122] The following examples are intended to illustrate but not limit the invention.

EXAMPLES

Example 1. Efficacy and Safety of Once Weekly Dalbavancin In Deep Skin and Soft Tissue Infections

[0123] This randomized, controlled study evaluated the safety and efficacy of two dose regimens of dalbavancin. Adult patients with skin and soft tissue infections (SSTI) involving deep skin structures or requiring surgical intervention were randomized to three groups: Study arm 1 received 1100 mg of dalbavancin via intravenous injection (IV) on day 1; Study arm 2 received 1g of dalbavancin IV on day 1 and 500 mg of dalbavancin IV on day 8; Study arm 3 received "standard of care." Clinical and microbiological response and adverse events were assessed.

<u>**Populations for Analysis**</u>

[0124] There were 62 patients randomized into the study; all received at least one dose of study medication. Four study populations were evaluated for safety and efficacy and were defined as follows: The intent-to-treat (ITT) population included all patients who received at least one dose of study drug (all randomized study subjects). The microbiological intent-to-treat (MITT) population were all ITT patients who had a culture-confirmed Gram-positive pathogen at baseline. The clinically-evaluable population were defined as those who 1) fulfilled all study entry criteria, 2) had no change in antimicrobial therapy for Gram-positive infection following Day 4, except for oral step-down therapy (only applied to standard of care group), 3) returned for the follow-up (FU) assessment visit (unless a treatment failure), and 4) did not receive a non-protocol approved concomitant antimicrobial (unless a treatment failure). The microbiologically-evaluable population was the subset of clinically-evaluable patients who had a culture-confirmed Gram-positive pathogen at baseline.

[0125] The study populations are shown in **Table 2.**

Table 2. Study Populations for Dalbavancin SSTI Treatment

Populations	Study arm 1 Dalbavancin 1100mg day 1	Study arm 2 Dalbavancin 1000mg day 1, 500mg day 8	Study arm 3 "Standard of care"
Randomized ITT	20	21	21
Treated	20 (100%)	21 (100%)	21 (100%)
Completed Study	18/20 (90%)	20/21 (95.2%)	21/21 (100%)
Clinically eval at EOT	16/20 (80%)	17/21 (81%)	21/21 (100%)
Clinically eval at FU	13/20 (65%)	17/21 (81%)	21/21 (100%)
MITT	14/20 (70%)	13/21 (61.9%)	14/21 (66.7%)
Micro eval at EOT	13/20 (65%)	11/21 (52.4%)	14/21 (66.7%)
Micro eval at FU	11/20 (55%)	11/21 (52.4%)	14/21 (66.7%)

ITT - intent-to-treat
MITT - subset of ITT population with culture confirmed Gram-positive infection
EOT - end of treatment
FU - follow up

[0126] The median age of the subjects was 50-55 years (range 18-86 years). There were no apparent differences in age across the treatment arms. There were differences in gender across treatment arms, but overall the study enrolled equal numbers of men and women. The patient population was predominantly Caucasian. These results were consistent for both the ITT and clinically evaluable populations.

[0127] 62 patients were enrolled, 20 in Arm 1 and 21 each in Arms 2 and 3. The most common comparators for standard of care were clindamycin, ceftriaxone, vancomycin and cefazolin. Mean duration of treatment in Arm 3 was 15 days.

Baseline pathogens and susceptibility

[0128] Of the 62 ITT patients, 66% (14 single-dose dalbavancin, 13 two-dose dalbavancin, 14 standard of care) had a pretherapy Gram-positive pathogen isolated (MITT population). The most common pathogen was *S. aureus*. The distribution of pathogens at baseline is shown in **Table 3**.

Table 3. Baseline Gram-positive Pathogens and Dalbavancin MIC Range for the MITT Population

Number with Pathogen (MIC)	Single dose Dalbavancin (1100 mg) (N=14)	Two-dose Dalbavancin (1000/500 mg) (N=13)	Standard of Care Regimens (N=14)
All *S. aureus*	13 (0.12)	11 (0.12)	10 (0.016-0.25)
Methicillin-sensitive	7	6	8
Methicillin-resistant	6	5	2
Group B streptococcus	0	2 (0.016)	2 (0.016)
Streptococcus pyogenes	0	1 (0.016)	1 (0.016)
Miscellaneous *Streptococcus* and nontypeable strains	3 (0.016)	2 (0.016)	4 (0.016)

__Clinical and microbiological responses__

[0129] The effectiveness of the three treatment regimens was determined by assessing the patients' clinical response and the documented or presumed microbiological responses. The primary efficacy endpoint was clinical response at the follow-up visit for the clinically evaluable population. Clinical response, for both EOT and FU visits, was categorized as success (cure or improvement) or failure (including indeterminate results). Patients classified as successes must not have received additional systemic antibacterial treatment for their infection. Failure was defined as persistence of one or more local or systemic signs and symptoms of SSTI such that treatment with new or additional systemic antibacterial agents was required for the SSTI.

[0130] Microbiological outcome, a secondary efficacy variable, was assessed in the subpopulation of patients who had microbiologically documented SSTI (*i.e.*, at least one identified baseline pathogen). A microbiologic response was assessed for each Gram-positive pathogen identified at baseline (*i.e.*, eradication, presumed eradication, persistence, presumed persistence). For patients for whom follow-up cultures were not performed, the microbiologic responses for baseline pathogens were presumed on the basis of the clinical response. Microbiologic response by patient at the EOT and FU visits was graded as success (*i.e.*, all Gram-positive organisms eradicated or presumed eradicated) or failure (*i.e.*, at least one Gram-positive organism persisted or presumed

to have persisted, multiple pathogens with partial eradication). At both the EOT and FU visits, colonization and superinfection were assessed. At the FU visit, a patient's bacteriological response could also include recurrence.

Clinical efficacy

[0131] Clinical success rates are shown in **Table 4**. In the clinically-evaluable population, 61.5% of patients in the single-dose dalbavancin, 94.1% in the two-dose dalbavancin, and 76.2% in the standard of care group were classified as successes at the time of the FU assessment. In an exploratory subanalysis of those patients categorized with deep or complicated SSTI at baseline, two-dose dalbavancin therapy also provided a higher clinical success rate (93.8%), compared with the single-dose dalbavancin and standard of care therapies, 58.3% and 73.7%, respectively.

[0132] Similar success rates at both the EOT and FU assessments were found in the supportive ITT and microbiologically-evaluable populations with a consistent trend towards a more favorable response following treatment with two-dose dalbavancin (**Table 3**). For the MITT population, clinical success rates at the FU assessment for those with methicillin-resistant *S. aureus* (MRSA) were 50% (3/6) for single-dose dalbavancin, 80% (4/5) for two-dose dalbavancin, and 50% (1/2) for patients treated with a standard of care regimen

Table 4. Clinical Success Rates by Analysis Population and Treatment Group

Population	Single-dose (1100 mg) Dalbavancin	Two-dose (1000/500 mg) Dalbavancin	Standard of Care Regimens
ITT at EOT	15/20 (75.0)	19/21 (90.5)	17/21 (81.0)
ITT at FU	12/20 (60.0)	19/21 (90.5)	16/21 (76.2)
MITT at EOT	10/14 (71.4)	12/13 (92.3)	10/14 (71.4)
MITT at FU	7/14 (50.0)	12/13 (92.3)	9/14 (64.3)
Clinically evaluable at EOT	13/16 (61.5)	16/17 (94.1)	17/21 (81.0)
Clinically evaluable at FU	8/13 (61.5)	16/17 (94.1)	16/21 (76.2)
Microbiologically evaluable at EOT	10/13 (76.9)	10/11 (90.9)	10/14 (71.4)
Microbiologically evaluable at FU	6/11 (54.5)	10/11 (90.9)	9/14 (64.3)

Microbiologic Efficacy

[0133] The success rates of the different treatment regimes with respect to different pathogens is shown in **Table 5**. For the microbiologically-evaluable population, eradication/presumed eradication rates at the FU assessment for all organisms were 58.3% (7/12) for single-dose dalbavancin, 92.3% (12/13) for two-dose dalbavancin, and 70.6% (12/17) for patients in the standard of care group. For isolates that persisted, there was no change in dalbavancin MIC. At FU, *S. aureus* eradication rates were higher for the two-dose dalbavancin group (90%) compared with single-dose dalbavancin (50%) and standard of care (60%) treatments. Similar findings were observed for the MITT population; two-dose dalbavancin eradicated 80% of MRSA isolates **(Table 5)**.

Table 5. Success Rates by Pathogen for Microbiologically ITT Population at FU Assessment

	Single-dose (1100 mg) Dalbavancin	**Two-dose (1000/500 mg) Dalbavancin**	**Standard of Care Regimens**
Total organisms	7/16 (43.8%)	14/16 (87.5%)	12/17 (70.6%)
All *S. aureus*	5/13 (38%)	9/11 (82%)	6/10 (60%)
Methicillin-sensitive	2/7 (29%)	5/6 (83%)	5/8 (63%)
Methicillin-resistant	3/6 (50%)	4/5 (80%)	1/2 (50%)
Miscellaneous streptococcus species	2/3 (67%)	4/4 (100%)	5/7 (71%)

[0134] For the microbiologically-evaluable and MITT populations, the microbiological success rates at EOT and FU are summarized in **Table 6**. Comparable microbiologic success rates were reported at both visits for patients treated with two-dose dalbavancin and standard of care regimens (approximately 64% to 77%), whereas those given a single dose of dalbavancin had lower rates of success (<40%). The microbiologic success rates at EOT/FU in the microbiologically-evaluable population paralleled clinical response findings: 38.5%/27.3% for single-dose dalbavancin,

72.7%/72.7% for two-dose dalbavancin, and 71.4%/64.3% for standard of care therapy. Similar findings were observed for the MITT population (data not shown).

Table 6. Microbiologic Success Rates

	Single-dose (1100 mg) Dalbavancin	Two-dose (1000/500 mg) Dalbavancin	Standard of Care Regimens
MITT population			
EOT	5/14 (35.7)	10/13 (76.9)	10/14 (71.4)
FU	3/14 (21.4)	9/13 (69.2)	9/14 (64.3)
Microbiologically evaluable population			
EOT	5/13 (38.5)	8/11 (72.7)	10/14 (71.4)
FU	3/11 (27.3)	8/11 (72.7)	9/14 (64.3)

Pharmocokinetic analysis

[0135] For patients randomized to the dalbavancin treatment groups, 5 ml of blood was obtained on Day 8 for determination of dalbavancin plasma concentrations. For patients randomized to receive a 500 mg dalbavancin dose on Day 8, blood was obtained just prior to administration of the second dose. Additional 5 ml blood samples were obtained on Days 10 and 24 for patients were randomized to the single-dose dalbavancin group and on Days 20 and 34 for those who received two doses of dalbavancin.

[0136] Dalbavancin plasma concentrations were determined using validated liquid chromatography and mass spectrophotometer methods. The lower limit of quantitation was 500 ng/ml for plasma.

[0137] Mean dalbavancin concentrations collected on Study days 8, 10, and 24 in the single-dose regimen were 31.1 ± 7.1, 25.2 ± 4.8, and 10.2 ± 3.5 mg/l (mean ± SD), respectively. Dalbavancin concentrations following the two-dose regimen on Study days 8 (prior to the second dose), 20, and 34 were 30.4 ± 8.2, 21.2 ± 10.0, and 9.0 ± 4.4 mg/l, respectively. As expected, all patients had dalbavancin concentrations of greater than 20 mg/l through the first week following the first dose, and levels above 20 mg/l were maintained for an additional week with an additional dose of 500 mg IV on day 8. Generally, minimum bactericidal concentrations are about 4 to 10 mg/l.

Safety Evaluation

[0138] Each patient who received at least one dose of study drug (ITT population) was evaluated for drug safety through monitoring of adverse events (AE), including abnormal clinical laboratory test results and vital signs. AE were rated by the investigator as to their severity (mild, moderate, severe, life-threatening), and by the relationship to the study drug (not related, unlikely related, possibly related, or probably related).

[0139] A summary of the AE data is presented in **Table 7**. The majority of adverse reactions (90%) were considered mild to moderate in severity. All serious adverse reactions (8 events in 5 patients) were unrelated to study drug treatment. Approximately 59% of all patients who reported at least one treatment-emergent AE (19 single-does dalbavancin, 16 two-dose dalbavancin, 21 standard of care) experienced an event that was categorized by the investigator as possibly or probably related to study drug. Specifically, drug-related AEs were reported in 11 (55%) single-dose dalbavancin, 10 (48%) two-dalbavancin, and 12 (57%) standard of care patients. The most frequently reported drug-related AE in both the dalbavancin and standard of care treatment groups were diarrhea and nausea. A summary of types of AEs observed for the different treatment groups is presented in **Table 8**.

[0140] No dalbavancin-treated patient discontinued treatment prematurely due to an AE. Three of 21 (14%) patients on a standard of care regiment discontinued treatment prematurely due to an AE, including one patient who developed urticaria on Day 1 which was probably drug related and two patients who had AE unrelated to study drug (superinfection with *P. aeruginosa* and elevated vancomycin trough level).

Table 7. Summary of Adverse Event (AE) Data

	Single-dose dalbavancin (N=20)	**Two-dose dalbavancin (N=21)**	**Standard of care (N=21)**
≥1 AE	95%	76.2%	100%
%AE severe	15%	9.5%	4.8%
≥1 AE possibly/probably related to treatment	55%	48%	57%
AE leading to discontinuation of study medication	0	0	14.3%
≥1 severe AE	2 (10%)	2 (9.5%)	1 (4.8%)

Table 8. Most Common Adverse Events

	Single-dose dalbavancin (N=20)	Two-dose dalbavancin (N=21)	Standard of care (N=21)
Diarrhea	20%	9.5%	28.6%
Nausea	10%	28.6%	9.5%
Hyperglycemia	5%	14.3%	19%
Limb Pain	10%	9.5%	9.5%
Vomiting	10%	14.3%	4.8%
Constipation	5%	4.8%	14.3%

Discussion

[0141] This open-label, randomized Phase II trial shows that dalbavancin is effective for the treatment of adults with SSTI. The majority of enrolled patients had deep or complicated infections (>90%) and infections that required surgical intervention (~70%), while approximately 45% had underlying diabetes mellitus.

[0142] Two weekly doses of dalbavancin had a numerically higher clinical response rate than either a single-dose of dalbavancin or the standard of care regimen. Data from both ITT and clinically-evaluable populations suggests that a regimen of two sequential weekly injectable doses of dalbavancin (1000 mg, 500 mg weekly) is effective in the treatment of SSTIs. The standard of care group was treated for a median duration of 13 days. At follow-up, 94% of clinically-evaluable patients treated with two-dose dalbavancin were considered clinical successes versus 76% of those given a standard of care regimen and 61.5% of patients receiving single-dose dalbavancin.

[0143] *S. aureus* was the most frequently isolated organism at baseline. In this trial, approximately 83% of patients were infected with *S. aureus* and 38% of all *S. aureus* strains were MRSA. Most infections (80%) were caused by a single pathogen. The MICs for dalbavancin against Gram-positive isolates, including MRSA, ranged from 0.016 to 0.25 mg/L.

[0144] Microbiological success rates paralleled those of clinical response for the clinically-evaluable population. For all organisms combined, treatment with the two-dose weekly dalbavancin regimen provided higher eradication rates at the 2 week post-therapy assessment (92%) compared with single-dose dalbavancin (58%) and standard of care therapies (71%). Overall, rates of *S. aureus* eradication were observed in 90%, 50%, and 60% of patients, respectively. For the MITT population, rates of eradication for MRSA were 80% for the two-dose dalbavancin regimen versus 50% for both the single-dose dalbavancin and standard of care therapies.

[0145] Concentrations of dalbavancin obtained at the end of the single-dose and two-dose weekly treatment periods (Day 10 or Day 20, respectively) were similar suggesting little drug accumulation following the second weekly dose. The higher rate of clinical success observed with the two-dose regimen is suggestive of time-dependent killing wherein sustained levels of drug or drug exposure were provided with two doses of dalbavancin separated by one week. Dalbavancin plasma levels measured at the end of the weekly dosing interval were substantially greater than the reported MIC_{90} for pathogens responsible for the majority of SSTIs (<0.03 to 0.5 mg/L), including those found in this trial. These levels were also above the minimum bactericidal concentrations of 4 to 10 mg/l.

[0146] The overall rate of adverse reactions was similar for both dalbavancin regimens and the standard of care group. Gastrointestinal drug-related adverse events (*i.e.*, diarrhea and nausea) were most commonly reported across the three treatment groups. The majority of these events were mild and self-limited. No dalbavancin-treated patient was withdrawn from the study early due to an adverse reaction, nor were any serious adverse events attributable to the glycopeptide reported, yet 14% of the standard of care group withdrew due to adverse effects. The novel dosage regimen thus had reduced adverse side effects in comparison to the standard of care. The data from this trial found no evidence that dalbavancin induces any degree of clinically significant hepatotoxicity or nephrotoxicity.

[0147] The two-dose dalbavancin regimen appears effective for treatment of patients with complicated SSTIs. Dalbavancin at both doses was well tolerated in this clinical trial, with an adverse event profile similar to that of the standard of care group.

Example 2. Pharmacokinetics and Renal Excretion of Dalbavancin in Healthy Subjects

[0148] The primary objectives of this study were to characterize the pharmacokinetics of dalbavancin and to calculate the extent of renal excretion in healthy subjects receiving a therapeutic dose of the drug. This was an open label, non-comparative, study.

Study Drug Treatment

[0149] Healthy male or female subjects between 18 and 65 years of age were administered a single 1000 mg IV dose of dalbavancin infused over 30 minutes.

[0150] Six subjects, one female and five male, were enrolled, received study medication, and completed all aspects of the study. Three subjects were Caucasian and three subjects were African-American. Mean age was 29.8 years (range 22 to 63). Mean height was 68.6 inches (range 63 to 75) and mean weight was 179.6 lbs (140 to 244).

Pharmacokinetics

[0151] Blood and urine (24-hr collections) were collected on study days 1, 2, 3, 4, 5, 6, 7, 14, 21, 28, and 42. Blood samples were drawn into heparinized tubes and centrifuged. Plasma was separated and stored frozen at -20°C until time of assay. Plasma and urine samples were assayed for dalbavancin using validated LC/MS/MS methods. The lower limit of quantitation of the assay was 500 ng/mL for urine and plasma.

[0152] Dalbavancin pharmacokinetic parameters were estimated by non-compartmental methods using the WinNonlin™ software (Pharsight Corporation). The peak concentration (C_{max}) values were obtained directly from the observed data. The area under the plasma concentration-time curve (AUC) was calculated using the linear trapezoidal rule. Clearance (CL) was computed as dose/AUC. The elimination half life ($t_{1/2}$) was estimated by linear regression of the log-linear portion of the log concentration versus time curve. Estimates of the volume of distribution (V_Z) was calculated using the regression parameters, while the volume of distribution at steady state (Vss) was calculated from the area under the first moment curve (AUMC) multiplied by the dose

and divided by AUC. The cumulative amount of dalbavancin excreted in urine was determined as the integrand of the urine excretion rate (AURC). CL_R, or renal clearance, was calculated as the ratio: CL_R = AURC/AUC.

[0153] Plasma concentrations of dalbavancin versus time are shown for all subjects in **Figure 1**. Pharmacokinetic parameters are presented in **Table 9**. Concentrations were similar across all subjects. Peak plasma concentrations were approximately 300 mg/L and were achieved immediately following the end of infusion. Dalbavancin shows an apparent volume of distribution of more than 10 L and is assumed to be well distributed in the extracellular fluid.

[0154] Dalbavancin was slowly eliminated with a $t_{1/2}$ of 9-12 days. The total drug clearance was 0.0431 ± 0.0074 L/hr. The estimated fraction of drug excreted unchanged into urine was 42% of the administered dose, and renal clearance was estimated as 0.018 L/h. The variability observed across subjects was low with a coefficient of variation of less than 22% across all pharmacokinetic parameters.

Table 9. Pharmacokinetic parameters

	Cmax mg/L	$t_{1/2}$ h	**AUC** mg.h/L	V_Z L	**CL** L/h	V_{SS} L	**AURC** mg	**% Renal Excretion**	CL_R L/h
Mean	301	257	23843	16.0	0.0431	11.5	419	41.9	0.0181
Sd	65	21	4526	3.1	0.0074	2.13	27	2.7	0.0036
CV%	21.6	8.1	19.0	19.5	17.1	18.6	6.4	6.4	20.1
Min	243	227	19844	11.7	0.0332	8.58	379	37.9	0.0130
Max	394	282	30100	19.6	0.0504	13.9	448	44.8	0.0226

Safety Assessments

[0155] Adverse events were recorded and assessed for severity and relationship to study drug. Laboratory data (chemistry panel, CBC with differential, urinalysis) were collected and assessed for changes from baseline and out-of-range values. ECG, physical examination, and vital signs were obtained, and changes from baseline were assessed.

[0156] Dalbavancin was well-tolerated in this study. No subject deaths or serious adverse events were reported during this study and no subject was prematurely withdrawn from study due to an AE.

[0157] All volunteers reported at least one AE, all of mild intensity. Three volunteers reported AEs that were possibly related to study medication: elevated ALT (value 46 IU/L, upper limit of normal 40 IU/L) in one subject; eosinophilia (value 0.5 x $10^3/\mu L$, upper limit of normal 0.4 x $10^3/\mu L$), elevated LDH (value 303 IU/L, upper limit of normal 90 IU/L), elevated ALT (value 54 IU/L, upper limit of normally 40 IU/L), elevated AST (value 42 IU/L, upper limit of normal 40 IU/L) all in one subject; and tinnitus in one subject.

[0158] No trends were seen for post-baseline hematology, chemistry, vital signs, and ECG results.

Discussion

[0159] A single 1000 mg IV dose of dalbavancin was well-tolerated. Following a single intravenous infusion of 1000 mg, plasma concentrations of dalbavancin above 45 mg/l are maintained for at least seven days. This is above concentrations known to be bactericidal (4-32 mg/l). This supports the use of dalbavancin as a once-weekly regimen. The urinary elimination profile indicates that renal excretion is an important elimination pathway, with approximately 40% excreted in urine. This finding is consistent with observations in animals. Since the kidneys are not the exclusive elimination route, a dosing adjustment for dalbavancin may not be necessary in renally impaired patients.

Example 3. Protein Binding of Dalbavancin using Isothermal Titration Microcalorimetry

[0160] Binding of dalbavancin to proteins was measured by isothermal titration microcalorimetry (ITC) in 20 mM phosphate, 150 mM NaCl, pH 7.4 at 25 and 37 °C using a Microcal VP-ITC instrument. In a typical experiment, 25 x 10 μl of protein (~150 μM) was injected into a calorimeter cell containing dalbavancin solution (~5 μM). Actual protein and dalbavancin concentrations were determined by measuring absorbence at 280 nm. Control experiments included injections of protein into buffer (in the absence of dalbavancin) to account for the heats of dilution of protein under identical conditions. For comparison, similar experiments with some necessary modifications were performed using teicoplanin.

[0161] Experiments with dalbavancin were conducted with each of the following proteins: human albumin; dog albumin; rat albumin; bovine albumin; and human α-glycoprotein.

Teicoplanin was studied with human albumin and α-glycoprotein. A comparison of binding affinities at two different temperatures is shown in **Table 10.**

Table 10. Comparison of apparent binding affinities (Ka, X 10^5 M^{-1})

	25 °C		37 °C	
Dalbavancin				
Human albumin	1.35	(± 0.2)	1.33	(± 0.15)
Rat albumin	3.1	(± 0.5)	2.8	(± 1.8)
Dog albumin	0.62	(± 0.09)	0.50	(± 0.13)
Bovine albumin	1.38	(± 0.14)		
α-glycoprotein	1.84	(± 0.36)	4.8	(± 2.3)
Teicoplanin				
Human albumin	0.96	(± 0.08)		
α-glycoprotein	0.07	(± 0.01)		

The ± errors quoted are the standard deviations obtained from the fitting routine.

[0162] Integrated heat effects, after correction for heats of dilution, were analyzed by non-linear regression using a simple single-site binding model with the standard Microcal ORIGIN software package. Raw data (μcal/sec) for each injection were integrated to give the total heat effect per addition, then divided by amount of injectant to give kcal/mole of injectant. The same integration was applied for control dilution effects, and this was subtracted from the actual titration data. This provided a differential of the binding curve in which the extent of binding is proportional to the total heat liberated (or absorbed). This was then analyzed by non-linear regression methods in terms of various standard binding models. The simplest model assumes simple non-competitive binding equilibrium, and gives three parameters:

K_a (=1/Kdiss) is the binding association (dissociation constant)

ΔH = the enthalpy of binding (the size of signal related to binding)

N = number of binding sites (assuming the binding model is correct)

[0163] Assuming non-competitive binding, N is the (relative) number of moles of injectant required to saturate all the available binding sites in the sample. For the dalbavancin experiments, dalbavancin is the "sample" and the protein (HSA, etc.) is the "injectant." These preliminary results indicate that the binding is relatively weak and, because of the poor solubility of dalbavancin, it is

difficult to determine the binding stoichiometry (N) unambiguously. However, as seen in **Figure 2**, in all cases, the data fits well with N<1 (i.e., less than one to one protein to dalbavancin). Consequently, a value of N=0.5 means that it only takes half as many moles of protein to bind all the dalbavancin than would be expected. In other words, each protein apparently binds two dalbavancin molecules. It is possible that dalbavancin forms a dimer that binds 1:1 with a protein. Results of binding stoichiometry modeling suggests that two dalbavancin molecules are bound to one molecule of protein, unlike teicoplanin, which exhibits 1:1 binding.

[0164] **Table 11** presents the calculated percent bound for antibiotic concentrations in the range 1-500 μM, assuming physiological concentrations of human serum albumin (6×10^{-4} M) and α-glycopeptide (1.5×10^{-5} M). To relate this to the clinical situation, the peak concentration of dalbavancin in man is approximately 300 mg/L, or 165 μM.

Table 11. Calculated percent Binding of Teicoplanin and Dalbavancin to Plasma Proteins

Concentration of antibiotic (μM)	Dalbavancin	Teicoplanin
Human albumin		
1	98.8	98.3
10	98.8	98.3
100	98.7	98.0
165	98.6	ND
250	98.5	ND
500	98.0	ND
Human α-glycoprotein		
1	73.0	9.6
10	68.2	9.1
100	26.2	6.0
165	16.9	ND
250	11.4	ND
500	5.9	ND

ND= Not done

[0165] In these experiments, the binding of dalbavancin to human serum albumin exceeds 98%. The fraction bound is fairly constant across the selected range of dalbavancin concentrations, i.e. 1-500 μM. This range encompasses the therapeutic concentrations in man. Binding of dalbavancin to α-glycoprotein is much greater than that of teicoplanin. Dalbavancin demonstrates high capacity

and low affinity for plasma proteins of different origin, with similar K_a values across proteins from all species tested. These results help to explain some of the unique pharmacokinetic characteristics of dalbavancin. The binding and formation of a 2:1 dalbavancin:protein complex also explains the prolonged half-life, and the apparent volume of distribution, which approximates extracellular water volume. The low affinity helps explain the observed *in vivo* activity, which greatly exceeds what would be expected for a compound with a free fraction close to 1%. The high capacity for plasma proteins helps to explain the relatively high plasma concentrations achieved in spite of poor solubility of the compound at physiological pH.

Example 4. Pharmacokinetic Attributes and Tissue Distribution of Dalbavancin in Rats

[0166] Two studies were performed in rats administered a single IV infusion of 20 mg/kg [^{3}H]-dalbavancin. Excreta and more than 40 different tissues were collected through 70 days post-administration, and the tissue distribution and pharmacokinetics of drug-derived radioactivity were determined.

[0167] HPLC-purified [^{3}H]-dalbavancin was used for these studies. Radiolabeled drug was produced via tritium exchange and purified by HPLC.

Rat mass balance study

[0168] Mass balance studies were conducted to determine the excretion pattern of dalbavancin following a single intravenous (IV) infusion of dalbavancin in male rats.

[0169] Fifteen male Sprague-Dawley rats received a single IV dose of ^{3}H-dalbavancin (20 mg/kg, 100 μCi/rat). Following dose administration, urine and feces were collected at 24 hour intervals to 14, 36, and 70 days after the dose (3 rats/final collection time). Water and methanol cage washes were also collected. Carcasses were analyzed at the end of the collection period. All samples were analyzed for total radioactivity content by liquid scintillation counting (LSC).

[0170] Following IV administration of ^{3}H-dalbavancin in rats, drug-derived radioactivity was eliminated in both urine (~ 2/3 of excreted radioactivity) and feces (~ 1/3 of excreted radioactivity). Approximately half of the radioactivity administered was eliminated in the urine and feces within the first week, which is consistent with a plasma $t_{1/2}$ of approximately 1 week. At 70 days post dose, only 4.5% of the dose remained in the carcass. Negligible radioactivity was

recovered in the cage washes. Virtually all of the administered radioactivity was accounted for (urine, feces, carcass, cage washes, and tritium exchange) during the study.

Rat quantitative tissue distribution (QTD) study

[0171] Quantitative tissue distribution studies were conducted to assess the tissue distribution of dalbavancin following a single IV infusion of dalbavancin to male rats.

[0172] Forty-one male Sprague-Dawley rats received a single IV infusion of ^{3}H-dalbavancin (20 mg/kg, 50 μCi/rat). Rats (3 per time-point) were euthanized at 12, 24, 48, 72, 96, 120, 144, 168, 336, 840, 1176 and 1680 hours post dose for collection of blood, plasma, and tissues (including carcass). All samples were analyzed by LSC.

[0173] Concentration-time profiles were determined for more than 40 tissues, including kidney, liver, spleen, blood, plasma, lung, and skin. Concentrations and $t_{1/2}$ values of drug-derived radioactivity in tissues, including skin, were comparable to those observed in plasma. Dalbavancin was found to be rapidly and extensively distributed with all tissues having quantifiable concentrations of drug-derived radioactivity within 12 hours after post-infusion. Most tissues reached maximum concentration (C_{max}) within 24 h after the dose. Recovered radioactivity after 5 days was <5% of the dose in any single tissue. By 70 days after the dose, only the carcass retained > 1% (2.34%) of the administered radioactivity. Thus, dalbavancin did not accumulate in any single tissue, organ, or blood cellular component. Concentrations of radioactivity in the CNS were low but detectable in this healthy animal model. Dalbavancin was found to penetrate the skin with concentrations of drug-derived radioactivity that were as high as or higher than in plasma. Blood to plasma ratio of drug-derived radioactivity remained relatively constant over time and was <1.

[0174] As part of the QTD studies, bile samples were collected from bile duct cannulated rats (4 animals) through 384 h (16 days) post-dose. Almost 11% of the dose was recovered in the bile over 384 h after the dose. This represents the majority of the drug-derived radioactivity found in feces.

Example 5. Quantitative determination of dalbavancin in plasma by HPLC-MS/MS

[0175] A HPLC-MS/MS method was developed for quantitative measurement of dalbavancin in plasma, as described below.

Preparation of dalbavancin calibration and quality control standards

[0176] Stock solutions of dalbavancin were prepared by dissolving dalbavancin in deionized water to prepare a 1000 μg/ml solution, followed by serial dilutions in deionized water to prepare 500, 50 and 10 μg/ml solutions.

[0177] Calibration standards of 100, 60, and 40 μg/ml dalbavancin concentration were prepared by spiking human plasma with appropriate volumes of a 1000 μg/ml dalbavancin stock solution prepared as described above. Calibration standards of 20 and 10 μg/ml concentration were prepared by spiking human plasma with appropriate volumes of a 500 μg/ml dalbavancin stock solution, and a calibration standard of 0.5 μg/ml was prepared by spiking human plasma with an appropriate volume of a 10 μg/ml stock solution.

[0178] Quality control standards of 90 and 30 μg/ml dalbavancin were prepared by spiking human plasma with an appropriate volume of a 1000 μg/ml dalbavancin stock solution prepared as described above. A quality control standard of 1.5 μg/ml was prepared by spiking human plasma with an appropriate volume of a 50 μg/ml solution.

Preparation of internal standard working solution

[0179] A 30 μg/ml working solution of internal standard BI-K0098, which is the diethyl-amino-propyl-amino derivative of A-40926, was prepared as follows. Approximately 10mg of BI-K0098 was dissolved in approximately 10 ml of mobile phase A (80% of 10mM Ammonium Formiate/Formic Acid, pH 3 (v/v), 10% of Acetonitrile (v/v), and 10% 2-Propanol (v/v)) to make a 1000 μg/ml internal standard stock solution. The stock solution (300 μl) was then diluted to a volume of 10 ml with mobile phase A to make a 30 μg/ml internal standard solution.

Preparation of samples for analysis

[0180] Samples were prepared as follows for quantitative determination of dalbavancin concentration in plasma. To 50 μl of calibration or quality control standards prepared as described above, 100μl of internal standard working standard solution was added and mixed. The mixture was permitted to equilibrate for five minutes at room temperature, followed by addition of 250 μl of acetonitrile. The mixture was then vortexed for 10 seconds, followed by centrifugation for 1 minute at about 10,000 rpm on an ALC micro-centrifugette 4214. Supernatants were transferred to clean

tubes and evaporated to dryness in a Savant Speed-Vac System at about 40 °C. Samples were then resuspended in 150 μl of mobile phase A.

Analytical Method

[0181] 50 µl samples prepared for analysis as described above were injected into a Phenomenex Jupiter C_{18} column (50 x 2 mm, C_{18} 5μm 300 A), and analyzed under gradient HPLC conditions at a flow rate of 0.3 ml/min. The gradient conditions were: initial, 80% mobile phase A/20% mobile phase B (20% 10mM Ammonium Formiate/Formic Acid, pH 3 (v/v), 40% Acetonitrile (v/v), 40% 2-propanol (v/v)); 1 minute, 20% mobile phase A/80% mobile phase B; 2 minutes, 20% mobile phase A/80% mobile phase B; 2.5 minutes, back to initial conditions.

[0182] The HPLC system was coupled to a PE SCIEX API-2000 triple quadrupole mass spectrometer, with turbo ion spray operating in a positive ionization mode. Air was used to generate a spray in the ion source. Probe temperature was set at 500° C with nitrogen as curtain gas. Multiple reactions monitoring (MRM) was employed using nitrogen as collision gas. The analytes were detected by monitoring the following ion transitions: 909.3 Da → 1429.3 Da for dalbavancin, and 923.3 Da → 1457.3 Da for the internal standard (BI-K0098). To avoid mass spectrometer contamination, a post-column flow diversion in the first minute and 2.5 minutes after the beginning of the chromatographic run was performed.

[0183] Software Sample Control 1.4 was used for the acquisition of data analysis and software MacQuan 1.6 was used for the integration of chromatographic peaks and statistical data evaluation.

Calibration curves

[0184] Linearity of the assay method was assessed byassaying calibration standards to generate a calibration curve. The concentration of dalbavancin in a plasma sample was determined by calculating the peak area ratio between dalbavancin and the internal standard.

[0185] Calibration curves for dalbavancin concentrations over an analytical range of 0.5-100μg dalbavancin/ml of human plasma were constructed using the equation $y = A + Bx$ (weighted 1/x), where A represents intercept of the curve, B represents the slope of the curve, x represents the dalbavancin concentration of calibration standard (μg/ml), and y represents the peak area ratio of dalbanvancin to internal standard. Three separate calibration curves were constructed. The results showed that dalbavancin/internal standard area ratio and dalbavancin concentrations varied linearly

over the analytical range. The lower limit of quantitation (LLOQ) was 0.5 μg dalbavancin per ml of human plasma. The slopes for the calibration curves were reproducible and their correlation coefficients were greater than 0.9995.

Stability of dalbavancin in plasma

[0186] The stability of dalbavancin in plasma samples was tested by analyzing three replicates quality control standards of human plasma samples, prepared as described above, at two different concentrations, 1.5 and 90 μg/ml. Detectable dalbavancin concentration was stable after three cycles of freeze-thaw treatment. Dalbavancin concentration in processed samples was stable after 24 hours at room temperature. No reduction in dalbavancin concentration with respect to time zero samples was observed.

Example 6. Dalbavancin Mass Spectroscopy Analysis.

[0187] The nature of dalbavancin multimers in solution was investigated and the conditions influencing the population ratio of dalbavancin multimer to dalbavancin monomer were determined by electrospray ion mass spectroscopy (ESI-MS).

[0188] Experiments were performed using an Applied Biosystem API III+ mass spectrometer equipped with a TurboIonSpray source, a Triple Quadrupole analyzer, operating in positive ion mode. The optimized conditions are reported in **Table 12** below.

Table 12: Instrumental Conditions for Dalbavancin Analysis on Applied Biosystem API III+ Mass Spectrometer.

IonSpray source:		**MS analyzer:**	
IonSpray voltage	5000 V	Interface plate voltage	650 V
Orifice plate voltage	80 V	Q0 road offset voltage	40 V
Curtain gas flow	0.6 L/min	Q1 park mass	1000
Nebulizer gas flow	1.2 L/min	Q1 resolution	120.8
Liquid flow	5 μL/min	Q1 delta mass	0.2
Interface heater	60°C	Q1 road offset voltage	27 V
		Lens 7 voltage	-50 V
		Q2 road offset voltage	-50 V
Scan conditions		Q3 park mass	1000
(Q1 scan):		Q3 resolution	110
		Q3 delta mass	0
Step	0.1 amu	Q3 road offset voltage	-70 V

Dwell time	1 msec	Lens 9 voltage	-250 V
		Faraday plate voltage	-250 V
		Channel electron multiplier voltage	-3800 V

Dalbavancin in Solution

[0189] Instrumental parameters were tuned on a dalbavancin solution containing 0.1 mg/ml of dalbavancin dissolved in a 8:2 water:isopropanol solution. A spectrum of dalbavancin in solution was acquired in the range of 500-2000 amu following direct injection of the solution. The resulting spectrum, as seen in **Figure 3**, indicates the presence of dalbavancin multimers. As a non-limiting example, one trace of the spectrum is attributable to a homomultimer of B_0, which is present as a $(2n\mathrm{M} + y(^{+}3))$ ion species, where n is a positive integer indicating the multiplicity of the homomultimer, *e.g.*, $n = 1$ when the multimer is a homodimer and $n = 2$ when the multimer is a homotetramer, M indicates the mass of the monomer, $y = n$ and $^{+}3$ indicates a plus three ion charge. For example, the homodimer of B_0 is provided when $n = 1, y = 1$, and M = mass of B_0. This homodimer species is assigned to a $(2\mathrm{M}\,^{+}3)$ ion trace in the mass spectrum.

Influence of Dalbavancin Concentration on the Population Ratio of Dalbavancin Multimer to Monomer:

[0190] The influence of dalbavancin concentration on the population ratio of multimer to monomer was evaluated by mass spectroscopy using the conditions described above. The spectra were acquired by direct infusion of dalbavancin solutions at concentrations of 20, 40, 60, and 80 μg/mL. The intensities of the main peaks were reported as a function of dalbavancin concentration and the population ratios of dalbavancin multimer to monomer were determined, as shown in **Figure 4**.

[0191] The data indicate that the population ratio of dalbavancin multimer to dalbavancin monomer increases with increasing concentration. This may help to explain the high drug loading capacities that may be administered to an individual. The role of multimer as a depot of monomer may decrease the tendency of higher concentration samples to form precipitates and enhance the concentrations which may be administered to an individual. The presence of multimers may also allow rapid administration of a dose of dalbavancin to an individual.

[0192] A non-limiting example of a method of determining the population ratio of dalbavancin multimer to monomer is provided, for example, by determining the ratio between peak intensities of

ions A and B as shown in **Figure 3**. Dividing the intensity of peak A by the intensity of peak B provides one measure of the population ratio of dalbavancin multimer to monomer.

<u>Influence of pH on the Population Ratio of Dalbavancin Multimer to Monomer</u>

[0193] The influence of solution pH on the population ratio of dalbavancin multimer to monomer was evaluated at the instrumental conditions described above and at the following solution pH values: 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, and 5.5. The population ratio of dalbavancin multimer to monomer was determined at each of the pH values and plotted against pH, as seen in **Figure 5**. It was determined that the population ratio of dalbavancin multimer to monomer increases with increasing pH.

[0194] While not to be limited to theory, it is believed that ionic groups, such as a carboxylate group on a first dalbavancin monomer, aid in the stabilization of dalbavancin multimers by forming ionic interactions with oppositely charged ions, such as tertiary nitrogen groups, on a second dalbavancin monomer. Such ionic interactions can be influenced by pH. It is believed that the increasing tendency of dalbavancin to be present as a multimer at higher pH is indication that ionic interactions are important in multimer stabilization. In particular, it is believed that dalbavancin multimers are destabilized at lower pH, presumably due to interruption of the ionic interactions contributing to multimer stability as certain functional groups, such as carboxylate groups, may be protonated at lower pH values.

<u>Influence of Solution Ionic Strength on the Population Ratio of Dalbavancin Multimer to Monomer</u>

[0195] The influence of solution ionic strength on the population ratio of dalbavancin multimer to monomer was determined by mass spectrometry. The mass spectra were obtained in electrospray positive mode on a Finnigan LCQ^{Deca} ion trap instrument previously tuned and calibrated in electrospray mode using Ultramark 1621, caffeine and MRFA (L-metionyl-arginyl-phenilalanyl-arginine). All the mass spectra were recorded using the conditions listed in **Table 13**. The sample parameters that were investigated are listed in **Table 14.**

Table 13. MS Conditions

Sample Inlet Conditions:	
Capillary Temperature (°C):	200
Sheat Gas (N_2, arbitrary units):	40
Sample Inlet Voltage Settings:	
Polarity:	positive
Source Voltage (kV):	4.70
Capillary Voltage (V):	34
Tube Lens Offset (V):	-60
Full Scan conditions:	
Scan range (amu):	500-2000
Number of microscans:	3
Maximum ion time (ms):	200
Zoom Scan conditions:	
Scan range (amu):	1218-1228
Number of microscans:	5
Maximum ion time (ms):	50

Table 14. Sample Parameters

Sample	μg/mL	Solvent	pH
Dalbavancin	100	$COO^-NH_4^+$ 5 mM	5
	100	$COO^-NH_4^+$ 50 mM	5
	100	$COO^-NH_4^+$ 100 mM	5

[0196] Sample water solutions were infused at 10 μL/min via a Harward syringe pump and the mass spectra were obtained as seen in **Figures 6-8**.

[0197] The obtained spectra indicate that the population ratio of dalbavancin multimer to monomer is influenced by by ionic strength. An increase in buffer concentration was found to correspond to a decrease in multimer mass traces and hence a decrease in dalbavancin multimer to monomer population ratio.

[0198] As mentioned, it is believed that ionic interactions are important in dalbavancin multimer stability. The fact that increasing ionic strength was correlated with decreasing intensity of multimer mass traces substantiates the role of ionic interactions in multimer stability. However,

as multimer mass traces were present even at higher ionic strengths, another, second, interaction may be involved in multimer stabilization.

[0199] While not bound to any theory, it is believed that hydrophobic interactions are important in stabilizing the multimer species of dalbavancin. If the stabilization of these non-covalent dalbavancin multimers was solely due to ionic interactions, it would be expected that an increase in ionic strength would result in total loss of multimer mass species. That is, it would be expected that as the ionic strength of the solution increases, the ionic interactions stabilizing the multimer would be disrupted by the increased population of ions in solution, with which the monomers would more readily associate. Consequently, the solution ionic strength would drive the multimers to disassociate into monomer components and the resulting mass spectra would be free of any multimer mass traces. However, even at high solution ionic strength (such as 100 mM ammonium formate), the presence of dalbavancin multimers is detected in the mass spectrum. Accordingly, the multimers of dalbavancin are deemed to be stabilized, at least in part, by hydrophobic interactions.

Structurally Similar Compound

[0200] It is believed that the improved efficacy of Dalbavancin is due at least in part to its ability to form multimers. It is thought that this unique characteristic is not shared even by very structurally similar compounds. A compound with a chemical structure similar to Dalbavancin was investigated by mass spectroscopy analysis for its ability to form multimers. The mass spectra were obtained in electrospray positive mode on a Finnigan LCQ^{Deca} ion trap instrument previous tuned and calibrated in electrospray mode using Ultramark 1621, caffeine and MRFA (L-metionyl-arginyl-phenilalanyl-arginine). All the mass spectra were recorded using the conditions listed in **Table 15.** The sample parameters that were investigated are listed in **Table 16.** Sample water solutions were infused at 10 μL/min via a Harward syringe pump and mass spectra were obtained as seen in **Figures 9 and 10.**

Table 15. Mass Spectra Conditions

Sample Inlet Conditions:	
Capillary Temperature (°C):	200
Sheat Gas (N_2, arbitrary units):	40
Sample Inlet Voltage Settings:	
Polarity:	positive
Source Voltage (kV):	4.70
Capillary Voltage (V):	34
Tube Lens Offset (V):	-60
Full Scan conditions:	
Scan range (amu):	500-2000
Number of microscans:	3
Maximum ion time (ms):	200
Zoom Scan conditions:	
Scan range (amu):	1218-1228
Number of microscans:	5
Maximum ion time (ms):	50

Table 16. Sample Parameters

Sample	**μg/mL**	**Solvent**	**pH**
Teicoplanin	50	H_2O	n.a.
	100	H_2O	n.a.

n.a. = not adjusted

[0201] A similar glycopeptide antibiotic (teicoplanin) does not show multimeric complexes in solution at various concentrations. This supports the indication that structurally similar compounds fail to form multimeric species in solution, and that this phenomenon may play an important role in the activity of the dalbavancin.

Example 7. Matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry of protein-dalbavancin complexes

[0202] 10 ul HSA, 0.150 mM was mixed with 10 ul dalbavancin solution (from 0.075 mM, 0.15 mM, 0.3 mM and 1.5 mM) and incubated for 60 min at 37°C. The samples were prepared for analysis using the dried droplet technique. Spectra were obtained on a BRUKER FLEX III, tof mass spectrometer previously tuned and calibrated using standard bovine serum albumin, acquiring and averaging spectra generated by 200 laser shots. Matrix: 9 parts of DHB-9 (2,5-dihydroxy-

benzoic acid) saturated in acetonitrile/H_2O (50:50), 1 part of sinapinic acid saturated in acetonitrile/H_2O (50:50). 0.5 ul of sample solution and 0.5 ul of matrix solution were mixed and placed on the laser target. [00179]

[0203] Dalbavancin binds to the protein as the monomer (1 HSA + 1 dalbavancin). At very high dalbavancin:protein ratios (1:2, 1:10), the presence of complexes containing 2 molecules of dalbavancin per protein molecule can be observed.

Example 8. Isothermal Titration Calorimetry of Binding of Dalbavancin to N,N'-diacetyl-Lys-D-Ala-D-Ala in the presence of Human Serum Albumin

[0204] The binding of dalbavancin to N,N'-diacetyl-Lys-D-Ala-D-Ala, a peptide analog of cell-wall targets of dalbavancin, was investigated by isothermal titration calorimetry (ITC) in the presence of HSA over a range of concentrations (up to 600 μM) at 25°C, with some additional measurements at 37°C. HSA increased the solubility of dalbavancin and reduced its binding affinity for the tri-peptide ligand. Results were compared with those for vancomycin. The observed effects plateaued at relatively low HSA concentrations, consistent with a non-competitive binding model that allows binding of ligand to dalbavancin both free in solution and (more weakly) to the dalbavancin-HSA complex.

[0205] Preliminary experiments demonstrated that, in the absence of serum proteins, dalbavancin and vancomycin show similar binding profiles: both give exothermic binding to N,N'-diacetyl-Lys-D-Ala-D-Ala, but no evidence of binding to dipeptide (D-Ala-D-Ala) or to Lys-D-Ala-D-Lactate. For dalbavancin/tri-peptide interaction, the data were consistent with binding with K_{diss} = 1-10 μM, depending on temperature, similar to vancomycin under the same conditions. In the presence of HSA, the solubility of dalbavancin is significantly increased and the binding affinity for tripeptide is reduced in a manner consistent with competitive or non-competitive binding by HSA for the antibiotic. The experiments described in this Example were designed in order to: (a) compare dalbavancin/tri-peptide measurements at different temperatures (25 and 37°C) and different HSA concentrations; (b) use these data to construct a binding model to compare with observed numbers.

[0206] Dalbavancin was supplied by Biosearch Italia. Other reagents were from Sigma: vancomycin hydrochloride (Sigma V-2002, fw 1485.7), N,N'-diacetyl-Lys-D-Ala-D-Ala (Sigma D-9904, fw 372.4), human albumin (HSA; Sigma A-3782; mw 69,366).

[0207] Antibiotics and peptides were dissolved in aqueous buffer (20mM Na phosphate, 150mM NaCl, pH 7.4) containing HSA, with gentle stirring immediately before each experiment. Peptide concentrations were determined by weight. Dalbavancin concentrations were determined either by weight or by UV absorbance using the molar extinction coefficients $\varepsilon = 12430$ (dalbavancin, $A_{280}^{1\%} = 68.42$), $\varepsilon_{280} = 6690$ (vancomycin). HSA concentrations were determined by UV absorbance (HSA, $\varepsilon_{280} = 37,700$; $A_{280}^{1\%} = 5.44$). Spectra were recorded at room temperature in 1cm pathlength quartz cuvettes using Shimadzu UV-160A or UV-1601 spectrophotometers, with samples diluted quantitatively with buffer if necessary to give absorbance in the 0.1-1 A range.

[0208] Isothermal titration calorimetry was performed using a Microcal VP-ITC instrument at 25 °C and 37 °C using standard operating procedures. See, *e.g.*, Wisemanet al., *Anal. Biochem.* (1989) 179, 131-137; Cooper, *et al.*, *Philos. Trans. R. Soc. Lond. Ser. A-Math. Phys. Eng. Sci.* (1993) 345, 23-35; Cooper, A, Isothermal Titration Microcalorimetry in C. Jones, B. Mulloy and A. H. Thomas (Eds.), Microscopy, Optical Spectroscopy, and Macroscopic Techniques. Humana Press, Totowa, NJ, (1994) p 137-150; Cooper, A., Microcalorimetry of protein-protein interactions in J. E. Ladbury and B. Z. Chowdhry (Eds.), Biocalorimetry: the applications of calorimetry in the biological sciences. Wiley, (1998) p 103-111; and Cooper, A., *Curr. Opin. Chem. Biol.* (1999) 3, 557-563. Samples were degassed gently prior to loading to prevent bubble formation in the calorimeter cell. Each experiment typically comprised 25 x 10 μl injections of peptide solution (≈ 1 mM) into the calorimeter cell (volume ≈ 1.4 ml) containing antibiotic solution (≈ 20-100 μM). Control experiments involved injection of ligand into buffer under identical conditions to determine heats of peptide dilution, and these values were used for correction of raw binding data prior to analysis. Dalbavancin/tri-peptide binding experiments were repeated several times at each temperature. ITC binding data were analysed using standard Microcal ORIGIN™ software to determine the apparent number of binding sites (N), the binding affinity ($K_{ass} = 1/K_{diss}$) and enthalpy of binding (ΔH).

TABLE 17

[0209] Thermodynamic data for binding of tri-peptide binding to dalbavancin and vancomycin determined by ITC assuming a simple non-cooperative binding model: effects of temperature and HSA.

	T °C	N	K_{ass} M^{-1}	Kdiss μM	ΔH kcal/mol	ΔS eu	[HSA] μM
Dalba-vancin	10	0.59	6.70E+05	1.49	-10.26	-9.60	0
	10	0.59	9.20E+05	1.09	-8.95	-4.30	0
	10	0.59	6.85E+05	1.46	-10.30	-9.60	0
	10	0.56	6.24E+05	1.60	-10.30	-9.90	0
	25	0.6	3.13E+05	3.19	-10.10	-8.90	0
	25	x	3.30E+05	3.03	-11.30	-12.60	0
	25	x	3.20E+05	3.13	-11.70	-14.00	0
	25	x	2.80E+05	3.57	-14.30	-23.00	0
	25	0.57	3.03E+05	3.30	-12.60	-17.00	0
	25	0.74	3.19E+05	3.13	-11.20	-12.50	0
	25	0.54	3.93E+05	2.54	-12.90	-17.60	0
with HSA	25	0.37	1.18E+05	8.47	-26.40	-65.50	13.6
with HSA	25	0.35	1.18E+05	8.47	-27.80	-70.10	13.6
with HSA	25	0.68	3.50E+04	28.57	-20.00	-46.20	34.2
with HSA	25	0.83	2.76E+04	36.23	-16.90	-36.50	80.7
with HSA	25	1.38	3.49E+04	28.65	-18.60	-41.70	104
with HSA	25	0.9	3.10E+04	32.26	-21.05	-50.00	288
with HSA	25	1.242	2.84E+04	35.21	-19.50	-44.90	430
with HSA	25	0.86	2.18E+04	45.87	-15.00	-30.40	601
	37	0.82	1.30E+05	7.69	-12.50	-16.80	0
	37	0.6	1.10E+05	9.09	-17.40	-33.20	0
with HSA	37	0.179	1.25E+04	80.00	-98.60	-299.00	482
with HSA	37	0.5	9568	104.52	-26.60	-67.60	516
Vancomycin	10	1.1	6.90E+05	1.45	-9.49	-6.80	0
	25	0.91	3.40E+05	2.94	-10.70	-10.60	0
	25	0.97	3.60E+05	2.78	-12.90	-17.80	0
with HSA	25	1.05	2.90E+05	3.45	-10.09	-8.80	513

[0210] ITC experiments on the binding of tri-peptide to dalbavancin in the absence of HSA give consistent data for binding affinities, with average K_{diss} in the region of 1.4, 3.1, and 8.4 μM at 10, 25, and 37 °C respectively (**Table 17**). Binding is exothermic. Concentration calculations here

indicate that N is closer to 0.5 under these conditions. This is consistent with the binding of one tri-peptide molecule per dalbavancin dimer under these conditions. These binding affinities and enthalpies of binding are comparable to those observed with vancomycin under the same conditions (**Table 17**, and D. McPhail, A. Cooper, *J. Chem. Soc.-Faraday Trans.* (1997) 93, 2283-2289.). Note also that vancomycin undergoes ligand-induced dimerization at higher concentrations.

[0211] Addition of HSA to the dalbavancin mixtures reduces the apparent binding affinity between tri-peptide and dalbavancin, though the binding is apparently more exothermic (**Table 17**). The HSA concentration dependence for this at 25 °C is shown in Figure **11**. After an initial rise (weakening) in apparent K_{diss} up to 35 μM HSA, it remains relatively constant for higher HSA concentrations approaching physiological levels (600 μM). The plateau level at high concentrations of HSA ($K_{diss} \approx 35$ μM) corresponds to around 10-12x weaker binding affinity than in the absence of HSA. A similar reduction is seen at 37 °C.

[0212] The HSA effect was not due to interaction with the tri-peptide. Control ITC experiments for binding of vancomycin to tri-peptide in the presence of HSA gave results comparable to those seen in the absence of HSA (see **Table 17**). This indicates that neither the peptide nor the closely related antibiotic, vancomycin, interact with HSA in solution. It follows that any effect of HSA on dalbavancin/tri-peptide interaction must be due to interaction between HSA and dalbavancin.

[0213] Although not wishing to be bound by theory, the above data are consistent with a non-competitive binding model. This model assumes that the tri-peptide ligand (L) can bind to dalbavancin (D) <u>both</u> in the free state and (possibly with different affinity) in the dalbavancin-HSA complex.

$$D + L \rightleftharpoons DL \quad ; \quad K_L = [D][L]/[DL]$$

$$D + HSA \rightleftharpoons D.HSA \quad ; \quad K_{HSA} = [D][HSA]/[D.HSA]$$

$$D.HSA + L \rightleftharpoons LD.HSA; \quad K_{LDHSA} = [D.HSA][L]/[LD.HSA]$$

[0214] Apparent (observed) ligand binding dissociation constant (non-competitive):

$$\begin{aligned} K_{app,L} &= [\text{total D}][L]/[\text{total DL complex}] \\ &= ([D] + [D.HSA])[L]/([DL] + [LD.HSA]) \\ &= K_L\{1 + [HSA]/K_{HSA}\}/\{1 + [HSA].K_L/K_{HSA}.K_{LDHSA}\} \end{aligned}$$

[0215] This shows a hyperbolic dependence of $K_{app,L}$ on free HSA concentration that agrees well with the observed data (Figure 11). At high [HSA] this reaches an asymptotic (plateau) value

$$K_{app,L} = K_{LDHSA} \text{ (for large [HSA])}$$

[0216] This suggests that the binding affinity for tri-peptide is around 35 μM when dalbavancin is bound to HSA, compared to 3 μM for free dalbavancin (25 °C figures).

[0217] Further mechanisms may be suggested in terms of whether dalbavancin is acting as a monomer or a dimer in its interactions with peptides or proteins. Direct comparison of dalbavancin with vancomycin (which shows unambiguous 1:1 binding at these low concentrations) shows that binding is complete at lower molar ratios (lower N) for dalbavancin (Figure 12). This is consistent with a 2:1 dalbavancin:peptide complex.

[0218] However, in the presence of HSA, the apparent N values increase (Table I17), and may be more consistent with 1:1 complexation. Although not wishing to be bound by theory, the model shown in Figure 13, showing the possible interaction of dalbavancin monomers and dimers with tri-peptide ligands and HSA, is consistent with these observations. Figure 13A depicts dalbavancin as in monomer-dimer equilibrium in solution (predominantly as dimer), but binding as monomer to two separate sites on HSA. This is consistent with the N = 0.5 values observed by ITC for binding of serum proteins to dalbavancin (Example 3). Figure 13B depicts ligand binding to the dalbavancin dimer in solution, and (more weakly) to the dalbavancin monomers attached to HSA. This is consistent with the non-competitive binding of dalbavancin to both tri-peptide and to HSA, with variable apparent stoichiometries.

[0219] In sum, this Example shows that HSA reduces the binding affinity of dalbavancin for tri-peptide ligand in a manner consistent with a non-competitive mechanism and that dalbavancin bound to HSA retains its ability to bind tri-peptide ligands, albeit with reduced affinity. These results are also consistent with a model in which dalbavancin is in monomer-multimer equilibrium, predominantly multimeric in solution, with strong affinity of multimers for peptide ligand. Dalbavancin monomers, both free and bound to serum albumin, may also bind peptides with a reduced affinity.

Example 9 A-40926 and Dalbavancin Preparations

Preparation of A-40926

[0220] A-40926, the natural glycopeptide produced by fermentation, was the starting material for producing Dalbavancin. It was produced by a *Nonomuria sp* ATCC 39727 as a mixture of A-40926 and its acetyl derivative (*see* U.S. Patent No. 4,935,238 and B. Golstain et al. *Antimicrobial Agent and Chemotherapy*, Dec. 1987, p.1961-1966). The acetyl derivative was first deacetylated to A-40926. After deacetylation, A-40926 was purified by column chromatography on polyamide as described below. The following description is representative of the current production method. The amounts reported here are about 1/4 of the amounts usually worked in an industrial preparation.

Deacylation of A-40926

[0221] 10 m^3 of fermentation broth (23 °C) containing a total of about 1 g/L of A-40926 and its acetyl derivative were adjusted, under stirring, at pH 11.4 with NaOH 30 %. Stirring was continued for 6 hours, then the temperature was reduced to 15 °C and the broth was microfiltered (Koch Protosep IV Microfilter with a 0.12 m^2 ceramic membrane 0.1μ). During microfiltration, water was continuously added to the retentate in order to obtain, at the end of the process, a permeate of 20-25 m^3 and a retentate of 4.5-5 m^3 (one half of the starting value).

[0222] The permeate, which contained A-40926, was analyzed by HPLC. When the deacetylation was completed, the pH of the permeate solution was adjusted at pH 7 with 30 % sulfuric acid (stored at 20 °C). In this example, 25 m^3 of filtered broth containing 6.62 Kg of A-40926 (268 mg/L) was obtained. The deacetylation yield was 66.2 %. If the microfiltration process was carried out for longer time and higher extraction volume than those employed in this process, the yield can be increased up to 90 %.

Purification of A-40926 on polyamide column

[0223] After extraction, A-40926 contained in filtered broth was purified on a polyamide column, as described below. The amount reported in this description is about 1/10 of the amount usually worked in an industrial preparation and is representative of the current production method.

[0224] 500 L of polyamide resin SC6 (from Macherey Nagel) were suspended in demineralized water and loaded into a column. The resin was then conditioned at pH 6-6.5 by eluting the column

with at least 2 BV (bed volume) of a buffer solution prepared by dissolving 4 Kg of sodium carbonate in 800 L of water and adjusting the pH of the resulted solution with acetic acid.

[0225] The A-40926 filtered broth (9000 L; assay 0.275 mg/L; A-40926 2475 g; pH 6±0.2; temperature 10±3 °C) was loaded into the column at about 5 g of activity per liter of resin (activity/resin ratio of 5-8 g/L was usually used). The column was washed with the following solutions: 3 BV (1500 L) of a solution at pH 6 prepared by dissolving 7.5 Kg of sodium carbonate in 1500 L of demineralized water and adjusting the pH with acetic acid; 4 BV (2000 L) of a solution at pH 8 prepared by dissolving 10 Kg of sodium carbonate in 2000 L of demineralized water and adjusting the pH with acetic acid; 1.5 BV (750 L) of a solution at pH 9 prepared by dissolving 4 Kg of sodium carbonate in 750 L of demineralized water and adjusting the pH with acetic acid.

[0226] A-40926 was recovered from the column by eluting with 4 BV (2000 L) of a buffer solution at pH 10 prepared by dissolving 10 Kg of sodium carbonate in 2000 L of demineralized water and adjusting the pH with acetic acid. The fractions containing purified A-40926 (concentration of A-40926 greater than 0.5 g/L and HPLC area % of the main component ($Bo+B_1$) greater than 80 %) were collected, neutralized with 1N HCl, and analyzed by HPLC. About 2000 L of final clear solution were obtained.

[0227] The resin used for the purification was regenerated with 1.5 BV of 1:1 mixture of isopropanol / 5 % NaOH followed by a washing with 5 BV of demineralized water.

A-40926 Concentration

[0228] The solution coming from the column was subject to several rounds of dilution/concentration steps to eliminate most of the inorganic salts in the solution. The solution was concentrated to 80 L by nanofiltration using a membrane with a cut-off of 250 D, diluted with 80 L of demineralized water, and re-concentrated at the starting volume (80 L) by nanofiltration. This operation was repeated at least 5 times. The pH of the final solution (80 L , pH 7.5) was adjusted at pH 6.3 with 23 % HCl. The solution was then diluted with 80 L of acetone, and its pH was adjusted again at pH 2.6 with 23 % HCl.

Decoloring

[0229] 680 g of charcoal PA 200 C (~0.3g/g A-40926) was added under stirring to the solution obtained in the above step (160 L). Stirring was continued for at least 30 minutes at room

temperature, then about 0.5-0.6 Kg of filter aid (DIF-BO) was added. The mixture was filtered through a filter cartridge. The clear solution obtained was concentrated under *vacuo* (45 °C) in order to reduce the acetone below 10 %. The final volume was about 100 L. The pH was then adjusted at 6.7 with aq. NaOH, and the concentration step was continued using the usual nanofilter until the A-40926 concentration was around 100 g/L. 20 L concentrated solution was obtained (A-40926 1884 g, 94.2 g/L).

Precipitation and drying

[0230] The previous solution was diluted under stirring with 20 L of acetone, and its pH was adjusted at 5.1 with 10 % HCl. To this solution additional 5 volumes of acetone (100 L) were added to complete the A-40926 precipitation. If water content was not <15% at this point, additional acetone was added. After 2 hours the suspension was centrifuged, and the solid was washed with 3 x 10 L of fresh acetone. The mother liquors were analyzed and discharged after having ascertained the absence of product.

[0231] Solid A-40926 was dried under reduced pressure at 30-35 °C in a static drier until the residual acetone was below 2 % and the water was less than 10 %. The product was then sieved through a 50 mesh sieve obtaining 2.08 Kg of purified A-40926 (HPLC assay 81.4 %; water 6.2 %; sulphated ashes 4.8 %). The yield, starting from the activity loaded on the column, was 68.4 %.

Synthesis of Dalbavancin

[0232] Dalbavncin (BI-397) was prepared from the natural glycopeptide A-40926 through a three-step synthesis as described in Malabarba and Donadio (1999), Drugs of the Future, 24(8):839-846. Specifically, A-40926 was first subject to an esterification step to make MA, which was then subject to an amidation step to make MA-A-1. A final hydrolysis step then converted MA-A-1 into dalbavancin.

Esterification step (Step 1)

[0233] The following description is representative of the current method in use.

Preparation of H_2SO_4 96%/MeOH (Solution A)

[0234] In a 15 L round bottomed flask equipped with a mechanical stirrer and a thermometer, 2.28 L of H_2SO_4 96% (~300 mL of H_2SO_4 96% per Kg of A-40926 powder) was added drop wise to 7.9 L of MeOH. An external ice bath was used to maintain the temperature between 0 and 5 °C.

<u>Reaction procedure</u>

[0235] Starting material A-40926 (7.6 kg; batch 019, assay 85.09 %; activity 6.46 kg; 3.73 mol) was suspended in a 140 L glass-lined reactor in MeOH (46 L), and the resulting suspension was cooled at 0 °C ± 2 °C. At this temperature the suspension was treated with the previously prepared Solution A (H_2SO_4/MeOH). The resulting solution was stirred at 0 °C for 22-26 hours while the reaction (a reaction aliquot diluted 100 times with 1:1 acetonitrile/water mixture) was monitored by HPLC analysis every two hours. The esterification was considered complete when the residual A-40926 was less than 5 % and diester was not more than 10 % as HPLC area %.

Ester (MA) isolation

[0236] When the reaction was complete, the mixture was cooled at -5 °C (+/- 2°C) and diluted with a same volume of cold water (54 L) maintaining the temperature below 5 °C. The product (MA) was precipitated by adjusting the pH of the solution at 5.5 (+/- 0.2) by slowly adding 10.2 L of triethylamine (TEA). Stirring was continued for an additional hour at 0-2 °C, then the solid obtained was centrifuged, washed with water (10 L per Kg of A-40926) and finally with MeOH (3 L of MeOH per Kg of starting A-40926) previously cooled at 10-15°C. Washing with water was done primarily to remove sulphates from MA.

[0237] Mother liquors and washings were separately analyzed and discharged if contained less than 1-2 % of activity. The product was dried *in vacuo* (50 mmHg) at 35-40 °C (external temperature) until the residual water was less than 10 %. 7.6 Kg of MA (5.63 kg activity, 3.23 mol) was obtained as a brownish powder.

[0238] The analysis showed the following values of HPLC area %: MA 89.8,

[0239] A-40926 3.2, Diester derivative 5.9. The HPLC assay was 74.2 %, activity 5.637 Kg; 3.23 mol; yield = 86.5 %.This material was used in the following step without any further purification.

Amidation step (Step 2)

[0240] The following description is representative of the current production method.

Preparation of the DMSO / HCl mixture (Solution B)

[0241] DMSO (1.6 L) was placed into a 10 L round bottomed flask, equipped with a mechanical stirrer and a thermometer, and cooled with an ice bath below 10 °C. HCl 37% (1 L) was then slowly added under stirring maintaining the temperature of the mixture below 25 °C.

Amidation procedure (production of MA-A-1)

[0242] Starting material MA 5.95 kg (assay 76.3 %, KF 8.9 %; 2.68 mol) was slowly dissolved under stirring in 19.2 L of 1:1 DMSO / MeOH mixture (~1.6 L DMSO and 1.6 L MeOH per Kg of MA powder) at room temperature. After 1 hour of stirring 709 mL of 3-(dimethylamino)-propylamine (DMEPA, MW 102.1; density = 0.812 g/mL; 5.63 mol; 1.96 mols per mol of starting MA) and 325 g of 1-hydroxybenzotriazole hydrate (HOBT·H_2O; MW 153.1; 2.04 mol; 0.71 mol per mol of starting MA) were added to the reaction mixture. Stirring was continued until a complete solution was obtained, then the mixture was adjusted at pH 3-3.1 (measured after diluting an aliquot of the reaction 10 times with water) by slowly adding about 2.0 L of Solution B (DMSO/HCl).

[0243] A dicyclohexylcarbodiammide (DCC) solution, prepared by dissolving 1.03 Kg of DCC (4.99 mol; MW 206.3; 1.74 mol per mol of MA) in 4.1 L of 1:1 DMSO/MeOH mixture, was added to the stirred reaction mixture in 10 minutes. Stirring was continued for 5 hours, then additional 51.5 g of solid DCC (0.25 mol) was added to the reaction mixture in order to lower the residual MA under 5%, maintaining the level of isoureas lower than 4-5 %. Isoureas are a group of by-products produced by further reaction of Dalbavancin with the excess of DCC.

[0244] Typically after 2 additional hours (7 hours total) the reaction was completed. At the end the mixture was diluted with water (60 L) to lower the DMSO concentration to 15 % (v/v) and adjusted at pH 2.3 with HCl 1N (0.85 L) to destroy any residual DCC.

Hydrolysis of MA-A-1 to Dalbavancin (Step 3)

[0245] After 30 minutes the mixture was adjusted at pH 12.0-12.1 with 15 % NaOH (8 L). Stirring was continued for 4 hours maintaining the mixture at this pH with small additions of NaOH 15%. After this time the residual MA-A-1 was less than 0.2 % as HPLC area %.

[0246] The mixture was then acidified at pH 3.0 with 1N HCl (19 L), and the suspension was filtered to remove the dicyclohexylurea formed. The solid cake was washed on the filter with demineralized water (2x20 L). Washings and filtrate were gathered together, obtaining a clear solution which was analyzed by HPLC. 152.8 L of solution containing 21.74 g/L of Dalbavancin (total activity 3322 g; 1.828 mol, yield = 68.2 %) was obtained.

1.1.1. B. Purification of Dalbavancin

[0247] The following description is representative of the current production method.

[0248] The 152.8 L of solution obtained from the hydrolysis step and containing 3322 g of Dalbavancin activity was split into two parts and each one was purified separately on the same chromatographic column containing 400 L of polyamide. In these two purification runs the activity/ resin ratio were 4.3 and 4.0 g/L respectively.

Polyamide Column Preparation

[0249] The glass-lined column (internal diameter = 40 cm, h = 320 cm) containing 400 L of polyamide resin was cleaned according to the resin regeneration procedures (see below) and conditioned with 2 BV (800 L) of demineralized water acidified with 4 L of AcOH (pH = 3.2).

Purification of the 1st portion

[0250] The first portion of 76.4 L of starting solution was diluted with H_2O (56 L) in order to lower the DMSO content under 5% (v/v), and acidified to pH 2.78 with 1 N HCl (3.4 L). This solution was then loaded onto the column at a flow rate of 150 L/h. After loading, the resin was washed with the following solutions: 4 BV (1600 L) of H_2O acidified with AcOH (8 L), pH = 3.2; 5 BV (2000 L) of AcONa 0.1 M, pH = 8.2; 1 BV (400 L) of H_2O acidified with AcOH (1 L), pH = 3.2. Dalbavancin was eluted with 4 BV (2400 L) of H_2O/MeOH (8:2) acidified with AcOH (6 L), pH = 3.4.

[0251] During the elution step, 22 fractions of 50-60 L each were collected and analyzed by HPLC. Fractions from 9 to 25 (concentration of Dalbavancin higher than 0.5 g/L and HPLC area % of (B_0+B_1) ≥80%) were pooled together, obtaining 969 L of solution with 1.56 Kg of Dalbavancin (yield = 93.9 %). This solution was then concentrated by nanofiltration, obtaining 125.7 L of solution with 1.38 Kg of Dalbavancin. 850 L of permeate, containing 145 g of impure Dalbavancin (8.7 %), were neutralized and discharged.

Resin regeneration

[0252] Before re-using the resin was washed with the following solutions: 2.5 BV (1000 L) of 1:1 MeOH /water acidified with acetic acid (2.5 mL/L); 2.5 BV (1000 L) of 1:1 0.5 % NaOH / isopropanol; 10 BV (4000 L) of demineralized water. The resin was then re-equilibrated with BV (800 L) of water acidified with acetic acid (2.5 mL/L).

Purification of the 2nd portion

[0253] The second portion of the starting solution coming from the hydrolysis step (76.5 L) was diluted with H_2O (56 L) to lower the DMSO content under 5% (v/v) and acidified to pH 2.87 with 3.0 L of 1 N HCl. The portion was then purified as previously described in the purification of the first portion. The pooled fractions (vol. = 972 L, Dalbavancin 1.54 Kg, yield = 92.7 %) were concentrated by nanofiltration, obtaining 133 L of solution with 1.46 Kg of activity. 850 L of permeate, containing 73 g of Dalbavancin (4.3 %), was discharged.

[0254] The concentrated solutions coming from the two purification steps were re-analyzed and pooled together giving 258 L of solution containing 2840 g of purified Dalbavancin . The purification yield was 86 %. The total yield, starting from MA, was 58.3 %.

Final polyamide regeneration

[0255] After the second purification run polyamide was regenerated with: 2.5 BV of 1:1 MeOH-water, acidified with AcOH (2.5 L) at pH =3.4; 2.5 BV of 1:1 0.5 % NaOH-isopropanol; 10 BV of demineralized water.

Decoloration and precipitation of Dalbavancin

[0256] 1.5 mol of 1N HCl per mol of Dalbavancin and 0.3 g of charcoal CG1 (0.85 Kg, from NORIT) per gram of Dalbavancin were added to the 258 L solution obtained above. The mixture was stirred at room temperature for at least 45 min. The pH was 3.1 . The suspension was then filtered on a SUPRA DISC cartridge mod. SDP-EK1 from SEITZ-SCHENK, and the cake was washed with 50 L of H_2O/MeOH 8:2. The filtrate was analyzed and concentrated again by nanofiltration, using a MPS 44 membrane with a cut off of 250 D. 21.3 L of concentrated solution containing 119 g/L of Dalbavancin (pH 4.1; MeOH 1.9 %,GC) was obtained. 909 mL of 1 N HCl

was finally added to adjust the pH at 2.63, which corresponds to the salification ratio of 1.65 $mol_{HCl}/mol_{Dalbavancin}$.

[0257] The solution (22.2 L) was poured out, under stirring, in 200 L of acetone. The solid obtained after decanting was centrifuged and washed with 14 L of fresh acetone. The product was then dried under reduced pressure (50 mmHg) at 35°C, maintaining the internal temperature under 30 °C for 17 hours. During the drying process, 1 L of pyrogen-free water (<250 EU/mL), divided in two portions of 0.5 L each, was sprayed on the solid after three and five hours in order to remove the residual acetone that otherwise is difficult to eliminate. The product was then sieved (50 mesh), obtaining 2592 g of Dalbavancin (HPLC Assay 82.4 %; water (KF) 14 %; Cl^- 3.0 %).

Example 10. Alternative Methods for A-40926 and Dalbavancin Preparation

[0258] The methods described below are alternative methods that can used in the A-40926 and Dalbavancin preparation process.

<u>A-40926 Preparation on XAD-7 HP</u>

Deacetylation and mycelium microfiltration

[0259] 150 L of fermentation broth containing A-40926 (pH 7) were stirred in a suitable reactor at room temperature (24 °C), and adjusted at pH 11.5 with a 2.5 N NaOH solution (2.5L). After 4 hours of stirring, the broth was adjusted at pH 10.6 with 15 % HCl, and microfiltered through a 0.2 micron membrane. 439 L of clear permeate was collected and then concentrated by nanofiltration using a MPS 44 membrane with a 250 D cut off. The A-40926 concentrated solution obtained (58.6 L; 3.89 g/L) was adjusted at pH 6.4 and stored at 4°C until used.

Column preparation and purification.

[0260] XAD-7 HP resin (8L) was suspended in a 1:1 water/methanol solution, filtered, and loaded into a proper glass-column (internal diameter 12 cm) with a peristaltic pump.

[0261] The resin was then washed with water and equilibrated with 6 BV of a sodium carbonate aqueous solution buffered at pH 6 prepared by dissolving 5 g of sodium carbonate per liter of water and adjusting the pH with acetic acid.

[0262] A portion of concentrated broth containing 194 g of A-40926 was loaded into the XAD-7 HP column. The resin was then washed with the following two buffered solutions, at a flow rate

of ½ BV /hour, in order to eliminate part of the hydrophilic and the colored substances present: 3 BV (24 L) of aq. 0.5 % Acetic Acid solution adjusted at pH 5 with 30 % sodium hydroxide; 5 BV (40 L) of a 8:2 mixture water/acetone with 5 mL of acetic acid / L of water.

[0263] A-40926 was finally eluted with 8 BV (64 L) of a 1:1 water/acetone mixture acidified with 5 mL of acetic acid / L of water. 16 fractions of 4 L each were collected. The rich fractions (from 5 to 15) in which A-40926 concentration was greater than 0.5 g/L were gathered together obtaining a solution containing 163.4 g of A-40926 (43 L, 3.8 g/L). The column yield was 81.3 %. The other fractions (200 L) containing 0.23 g/L (45.3g; 22.2 %) of less pure A-40926 were discharged.

[0264] After the elution the resin was regenerated with 6 BV (55 L) of NaOH 0.5% / isopropanol (1:1) mixture, and finally washed to neutral with 10 BV of water.

Charcoal treatment.

[0265] The collected fractions were adjusted at pH 2.5 with HCl 37% (70 mL) and then decolorized with 50 g charcoal type PA 200 (0.3 g/g of A-40926). The suspension obtained was stirred for 2 hours at room temperature and then filtered through a KS 50 filter (d=25 cm, time = 2.5 hours), obtaining 45.6 L of a slightly yellow A-40926 solution (3.5 g/L; yield=96.4 %).

Concentration.

[0266] The decolorized solution was adjusted at pH 7 with NaOH 30% (230 mL) and concentrated by nanofiltration and ultrafiltration. The use of these techniques was important for the elimination of the hydrophilic substances that were detected on the HPLC chromatograms at Rt = 2-4 minutes. When the retentate was concentrated to 1/10 of the starting volume (4 L), the same volume of water was added and the solution obtained was concentrated again. This concentration/dilution step was repeated three times in order to reduce the residual acetone to 0.25 %. The final solution (2.2 L, 146.3 g of A-40926, 66.5 g/L, yield=91.5%) was analyzed by HPLC. The purification yield was 75.4 %.

A-40926 crystallization.

[0267] A 300 mL portion of the A-40926 solution (19.9 g of A-40926) was further concentrated to 100 mL by using a laboratory scale ultrafilter and then heated at 60-65 °C. The pH of this solution was adjusted at 7 (30% NaOH), and 1.2 mL of 5:1 acetone/isopropanol mixture per mL of

concentrated solution was added drop wise at this temperature. The resulted mixture was left to cool at 20 °C. After 1.5 hours, the solid obtained was filtered, washed on the filter with acetone, and dried at 40 °C for 15 hours. 20.6 g of product (HPLC assay 82.0 %; A-40926 16.9 g) was obtained. The precipitation yield was 84.9 %. The overall yield, starting from the filtered broth, was about 64 %.

A-40926 Preparation on CG -71

Column preparation

[0268] CG-71 resin (350 mL) was poured into a glass-column (internal diameter = 4 cm) and washed with water. The resin was equilibrated with 3 BV of a sodium carbonate solution, prepared by dissolving 5 g of sodium carbonate in water at pH 6 with acetic acid. 250 mL fermentation broth (pH 7) containing 14.7g of A-40926 was loaded into the column (42 g/L resin). The resin was washed with the following three solutions: 1050 mL (3 BV) of aqueous solution of sodium carbonate (5 g/L) adjusted at pH 6 with acetic acid; 1750 mL (5 BV) of aqueous solution of sodium carbonate (5 g/L) adjusted at pH 8 with acetic acid; 3150 mL (9 BV) of aqueous solution of sodium carbonate (5 g/L) adjusted at pH 9 with acetic acid.

[0269] The activity was then eluted with 10 BV of demineralized water. 20 fractions of 500 mL each were collected. Fractions 12 to 15 were pooled together, obtaining 2.2 L purified solution containing 11.7 g of A-40926 (yield = 79.6 %). This solution was then concentrated by ultrafiltration and the concentrate solution was further diluted with demineralized water and ultrafiltered again. The solution obtained was further concentrated under reduced pressure to 50 mL.

A-40926 crystallization .

[0270] The concentrated solution was heated at 60 °C and treated under stirring with a 5:1 acetone/IPA mixture (60 mL). The mixture was then slowly cooled at room temperature. The solid obtained was filtered, washed with acetone on the filter, and dried under vacuum at 35 °C for 80 hours. 8.9 g of purified A-40926 (HPLC assay 84.2 %) was obtained. The overall yield was 51 %.

Alternative amidation step in dalbavancin synthesis using N-Methyl-2-pyrrolidine (NMP) as a solvent

[0271] MA mixture was added portion wise under stirring to a 1:1 NMP/MeOH mixture (64 mL). Stirring at 20-25 °C was continued until complete solution, then DMEPA (2.42 mL; 1.96

mol/eq_{MA}) and HOBT (1.06 g; 0.71 mol/eq_{MA}) were added. The pH of the reaction mixture (checked on a sample diluted 1:10 with water) was adjusted to 3.0 with 9.37 mL of 15% HCl in NMP (previously prepared from 34.0 mL HCl 37% dissolved in 57.7 mL NMP). Then a solution of DCC (3.17g; 1.57 mol/eq_{MA}) in NMP/MeOH 1:1 (12.7 mL) was added under stirring. The reaction was monitored by HPLC. The reaction was complete after about 6 hours (MA-A-1 88.9%, MA 7.3%, ISO 3.7%). This experiment suggests that NMP can be a convenient alternative to DMSO for the amidation reaction. The whole process was not influenced by this solvent change and the final Dalbavancin obtained was chemically equivalent to other batches.

Alternative method of Dalbavancin preparation: One-pot procedure

[0272] 10 g of A-40926 complex (HPLC titer 80.66%, 4.6 mmole) was suspended in 24 mL of MeOH under stirring at room temperature in a 100 mL glass reactor. The mixture was cooled at 0°C, and a solution of 4 g of HCl (g) in 16.4 mL of MeOH was added to complete the product solubilization. The temperature was then left to rise to 20°C while stirring was continued for additional 24 hours.

[0273] After this time, 40 mL of DMSO and 0.4 g of HOBT were added to the reaction mixture.

[0274] 1,1-dimethyamine propylamine was then added, adjusting the pH of the resulting reaction mixture between 3-3.1 (measured after diluting a sample 9:1 with water). 1.8 g of solid DCC was then added and stirring was continued for additional 15 hours. After this time the reaction mixture was transferred in a 1 L glass reactor and diluted with 80 mL of water. The pH was then brought to 12 by adding 240 mL of 15% NaOH. Stirring was continued for additional 60 minutes, and the mixture was acidified at pH 2.8 with 260 mL of 15% aq. HCl. About 800 mL of final clear solution containing 6.4 g of Dalbavancin was obtained (yield = 76%).

[0275] HPLC analyses showed that the profile of the product obtained is comparable with that obtained with the other manufacturing processes.

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[0276] Although the foregoing invention has been described in some detail by way of illustration and examples for purposes of clarity of understanding, it will be apparent to those skilled in the art that certain changes and modifications may be practiced without departing from the spirit and scope of the invention. Therefore, the description should not be construed as limiting the scope of the invention, which is delineated by the appended claims.

[0277] All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes and to the same extent as if each individual publication, patent, or patent application were specifically and individually indicated to be so incorporated by reference.